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Review

Cellular calcium in health and disease

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1. Introduction

Sidney Ringer, in 1882, was the first to recognize the relevance of calcium, an element discovered in 1908 [1], for cellular function. He discovered the necessity of Ca^{2+} for heart contraction and described its role during development of fertilized eggs and cell adhesion [2–4]. In the late 1920s, it became evident that intracellular Ca^{2+} is the link between hormone-receptor binding and cellular responses like secretion or movement [5–8]. Understanding the biological role of Ca^{2+} depended on methodological progress. First intracellular measurements of free Ca^{2+} using microinjection and the dye alizarin sulphonate [9] were controversial. An important breakthrough was the introduction of the Ca^{2+}-sensitive photoprotein aequorin by Ridgway and Ashley [10], and further improvements by metallochromic Ca^{2+} indicators [11]. The still rather limited sensitivity was overcome by Ca^{2+} microelectrodes [12] and by Ca^{2+}-sensitive fluorescent dyes, like indo-1 and fura-2 [13,14].

The clinical interest in Ca^{2+} was initially limited. It focused on changes in serum Ca^{2+} since hypocalcemia resulted in tetanic muscle spasms [15]. Later the hormonal regulation of serum Ca^{2+} level was investigated. The development of organic Ca^{2+} channel blockers created pharmacological tools which replaced the therapeutic regimens of many diseases, for example hypertension and arrhythmias [16,17]. Fleckenstein was the first to correlate the negative inotropic effect of Ca^{2+} antagonists to an inhibition of excitation–contraction coupling via a reduced Ca^{2+} entry [18]. This observation implied also a pivotal role of intracellular Ca^{2+} in pathophysiological situations.

To date, cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) has been involved in numerous important cellular functions, like growth and cell division [19–21], excitability [22], contraction [23], volume regulation [24,25], and exocytosis [26]. Resting levels of around 100 nM, some 10,000-fold below extracellular Ca^{2+} are generally observed. This disequilibrium is the result of a limited Ca^{2+} permeability of the plasma membrane, active Ca^{2+} extrusion processes and intracellular Ca^{2+} buffers [27]. Certain physical, electrical, or chemical stimuli evoke a Ca^{2+} response whereby levels of $[Ca^{2+}]_i$ of around 1 μM are reached. Calcium might exert its effect directly, via activation of certain ion channels, or indirectly via activation of additional mediators [28].

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This review will first summarize Ca^{2+}-transporting systems, intracellular Ca^{2+} buffers, and their integration under basal and stimulated conditions. Next, examples of disturbed Ca^{2+} homeostasis and of impaired Ca^{2+} signalling under pathophysiological conditions in various tissues are discussed. Finally, a pathophysiological concept is presented. The authors decided not to include the detailed role of Ca^{2+} in the programmed cell death, apoptosis, since this would exceed the scope of this review. For this topic the reader is referred to other publications [29–31]. Furthermore, specific problems related to transepithelial calcium transport were also not covered; they have been reviewed recently by several authors [32,33].

2. Cellular calcium homeostasis

$[Ca^{2+}]_i$ is a balance between Ca^{2+}-increasing, Ca^{2+}-decreasing, and Ca^{2+}-buffering mechanisms (Fig. 1). Although in the following these processes are presented separately, they are act hand-in-hand in adjusting $[Ca^{2+}]_i$ to the appropriate level.

2.1. Calcium increasing mechanisms

In addition to the Ca^{2+} gradient across the plasma membrane there exist Ca^{2+} concentration differences within the cell, since Ca^{2+} is sequestered in intracellular stores. $[Ca^{2+}]_i$ can be increased by two ways: release from internal stores or influx from the extracellular space. In both cases Ca^{2+} moves passively along its electrochemical gradient.

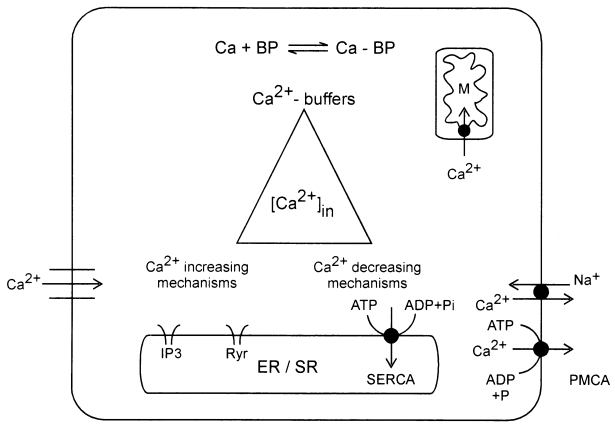

Fig. 1. Schematic diagram of cellular Ca^{2+} homoeostasis. Intracellular Ca^{2+} concentration is a result of the interplay of Ca^{2+}-increasing, Ca^{2+}-decreasing, and Ca^{2+}-buffering mechanisms. BP = binding protein, M = mitochondria, ER = endoplasmic reticulum, SR = sarcoplasmic reticulum, SERCA = Ca^{2+}-ATPase in SR and ER, PMCA = Ca^{2+}-ATPase in the plasma membrane.

2.1.1. Intracellular calcium stores

Ca^{2+} is stored in multiple ways [34]. Intracellular deposits of Ca^{2+} salt crystals are seen under pathological conditions, e.g., in atherosclerosis, pancreatic necrosis, nephrocalcinosis, etc. [35]. In vertebrate cells Ca^{2+} is usually stored as free or bound Ca^{2+} in intracellular pools with exchange rates that range from seconds to hours or days. Little is known about the slowly exchanging Ca^{2+} pools; these stores are most likely localized in mitochondria or acidic compartments like endosomes, lysosomes and the trans Golgi network [36].

Rapidly exchanging Ca^{2+} stores have been described in the microsomal fraction, that mainly consists of endoplasmic reticulum (ER), which could be stimulated by inositol-1,4,5-trisphosphate (IP_3) to release Ca^{2+} [34,37,38]. Using immunogold-labeling techniques, Ca^{2+} release channels and Ca^{2+} transport proteins have been detected close to the ER [39]. Thereby, the ER and its analog in muscle cells, the sarcoplasmic reticulum (SR), have been identified in most cells as the main Ca^{2+} pools [40]. There seem to be regional differences in Ca^{2+} storage properties of the ER, in some cells, specialized subcompartments, called calciosomes exist [41]. In chromaffin cells different spatial Ca^{2+} signals correlated to different localization of Ca^{2+}-ATPase-like proteins have been observed [42]. Furthermore, a nonhomogeneous distribution of IP_3 and ryanodine channels, of sarcoplasmic reticulum calcium pumps (SERCAs), and of the Ca^{2+} binding protein calsequestrin has been shown, suggesting different regions for Ca^{2+} uptake and Ca^{2+} release [42,43]. In cells possessing both IP_3 and ryanodine-sensitive Ca^{2+} channels a Ca^{2+} release after IP_3 stimulation does not prevent a further release via stimulation of the ryanodine receptor and vice versa. Pools of a different IP_3 sensitivity or of a different IP_3 receptor density seem to exist [44,45]. GTP and the cytoskeleton appear to organize and connect different IP_3-sensitive Ca^{2+} pools [46]. There exist further cell-type specific storage pattern in addition to IP_3 and ryanodine-sensitive Ca^{2+} pools [47–

51], enabling various cells to respond to physiological stimuli in an individual way [44].

The Ca^{2+} storage capacity of SR or ER critically depends on the intraluminal Ca^{2+} binding proteins calsequestrin and calreticulin [52]. Calsequestrin is the main intraluminal component of SR in skeletal and cardiac muscle [53,54], whereas calreticulin occurs mainly in smooth muscle SR and non-muscle ER [55,56]. Their intraluminal distribution varies from highly focused to homogeneous [57]. Both Ca^{2+} binding proteins (CaP) have a high capacity and contain low and high affinity Ca^{2+} binding sites [56], the low affinity Ca^{2+} binding sites bind Ca^{2+} with a K_D of about 1–2 mM [58].

There is evidence that cellular vesicles in neuronal or exocrine cells also contain calcium transport proteins as well as CaPs in their lumen [59]. In isolated pancreatic zymogen granules IP_3 and cyclic ADP-ribose (cADPR) have been shown to induce a Ca^{2+} release [60]; this release is integrated in the cellular Ca^{2+} signalling [46,61]. In pancreatic acinar cells the different Ca^{2+} storage compartments seem to be arranged like an intracellular tubule, enabling Ca^{2+} tunneling from the place of entry, usually the basolateral cell pole, towards the storage site at the apical cell pole [62].

2.1.2. Intracellular calcium release channels

For Ca^{2+} release from intracellular stores, two different channels, the IP_3 receptor-linked and the ryanodine receptor-linked channel, have been characterized [63–65]. Their structural and functional properties are presented in Table 1. The IP_3 receptor contains four subunits of around 320 kDa, each forming a tetramer. It is inserted in the ER membrane by six membrane spanning regions. Both, the N-terminus and the C-terminus of the protein are facing the cytosol, the latter contains the IP_3 binding region and the domains for Ca^{2+} and adenine nucleotide modulation. At least three genes code for the three different receptor types: Receptor types II and III share homology of 70 and 62%, respectively, with the type I receptor [66]. Furthermore, there exist various isoforms of the IP_3 receptor [63].

The functional importance of this IP_3 receptor heterogeneity and its role during tissue development or during pathogenic processes remains to be elucidated. The IP_3 receptor seems to be present in almost all vertebrate cells but the degree of expression varies. Olfactory cells [67] and lymphocytes [68] express another IP_3 receptor subtype in their plasma membrane whose nature and functional role remain hitherto unclear.

Binding of IP_3, which is produced by the G-protein-activated phospholipase C, triggers the release of Ca^{2+} from internal stores. There is an IP_3 binding site on every subunit, the number of IP_3 molecules required to open the channel is unclear [69–71].

Pharmacological inhibitors of the IP_3 channel are heparin and caffeine [72]. Protein kinase A, protein kinase C, calmodulin-dependent protein kinase II, as well as a G-protein-dependent mechanism modulate channel activity by phosphorylation [73,74]. IP_3-induced Ca^{2+} release at increasing Ca^{2+} concentrations follows a bell-shaped curve (maximum open probability at 0.2 μM) with positive feedback at lower Ca^{2+} concentrations and negative feedback at higher Ca^{2+} concentrations (above 0.5 μM) [75]. Finally, the IP_3-evoked Ca^{2+} release seems to be controlled by the Ca^{2+} content within the Ca^{2+} store [76].

Table 1
Intracellular Ca^{2+} channels

	IP_3 receptor	Ryanodine receptor
Molecular mass of subunit	320 kDa	560 kDa
Structure	tetramer	tetramer
Conductance	10–20 pS	100 pS
Activators	IP_3, Ca^{2+}	cADP-ribose, ryanodine (< 10 μM), Ca^{2+}, heparin, caffeine, imperatoxin activator (only skeletal form), adenine nucleotides
Inhibitors	heparin, Ca^{2+}, caffeine	ryanodine (> 10 μM), Ca^{2+}, ruthenium red, Mg^{2+}, imperatoxin inhibitor

The second important intracellular Ca^{2+} channel is the ryanodine channel [77–79]. The four subunits have a molecular weight of 564 kDa each. Around 95% of the protein towards the N-terminus are located in the cytosol, the other 5% comprise a membrane-spanning region with the C-terminus facing the cytosol, too. The number of transmembrane helices (4–12) is still controversial. The cytosolic part permits a close spatial relationship between the Ca^{2+} channel and the dihydropyridine (DHPR) receptor in the T-tubule of the striated muscle cell plasma membrane essential for stimulus contraction coupling [80].

There exist three isoforms of the ryanodine receptor: skeletal, cardiac, and brain/smooth muscle ryanodine receptors [81,82]. They are products of three different genes. Comparison of the amino acid sequences of the different tissue isoforms within one species revealed a homology of about 60–70%, which is lower than the homology of identical isoforms from different species (around 90%).

The ryanodine receptor is a cation-selective channel with high conductances for both monovalent (600 pS) and divalent (180 pS) ions. At 0.01–10 μM ryanodine stimulates Ca^{2+} release, higher concentrations (>10 μM) inhibit Ca^{2+} release [83], correlating with low and high affinity binding sites for ryanodine. Ryanodine receptors may be opened by depolarization, a property important for the functional coupling of the DHPR/ryanodine receptor complex.

The ryanodine receptor is also activated by $[Ca^{2+}]_i$ from 10 nM to 1 μM; this forms the basis of the calcium-induced calcium release (CICR). Higher intracellular Ca^{2+} concentrations ($>10^{-5}$ M) are inhibitory, suggesting various sites with different Ca^{2+} affinity in the cytosolic domains of the receptor [75]. The Ca^{2+} concentration within the Ca^{2+} store also affects Ca^{2+} release [84], probably mediated by calsequestrin. ATP and phosphorylation by Ca^{2+}-dependent calmodulin kinase stimulate [85], calmodulin and Mg^{2+} [86] exert an inhibitory effect [87]. Two scorpion toxins selectively act on ryanodine receptor subtypes, imperatoxin as inhibitor and imperatoxin as activator. The former inhibits ryanodine binding and activity of skeletal and cardiac ryanodine receptors; the latter activates the skeletal ryanodine receptor subtype [88].

In some cells the physiological effector molecule for the ryanodine receptor is cyclic ADP-ribose (cADPR) [89,90]. cADPR is generated from β-nicotinamide–adenine dinucleotide and activates the cardiac ryanodine receptor at concentrations of about 20 nM in sea urchin eggs [89]. In other cells higher doses of cADPR are necessary to induce Ca^{2+} release. Moreover, cADPR and calmodulin act synergistically during sensitization of CICR [91]. Therefore, the exact role of cADPR—messenger for or modulator of the ryanodine receptor—seems to vary among different cells [92].

Several proteins have been shown to be involved in the function of ryanodine receptors: the CaP annexin VI, S-100, calpain, and the immunophilin FK506 binding protein [93]. The physiological role of the latter is unclear. Upon binding of the immunosuppressive drugs FK506 or rapamycin the immunophilin dissociates from the ryanodine receptor thereby modulating its activity [94,95].

2.1.3. Plasma membrane calcium channels

Plasma membrane Ca^{2+} channels have been classified according to their gating mechanisms (Fig. 2) [37,39,96,97]. Ca^{2+} entry can be activated either by voltage changes, by binding of certain exogenous and endogenous transmitters or simply by membrane stretch. In most cells more than one type of Ca^{2+} influx pathway can be found [98].

According to their electrical behaviour and sensitivity to inhibitors the voltage-dependent Ca^{2+} channels (VOC) are classified into L-type (long lasting—large capacitance), T-type (transient), N-type (neither), and P-type. L-type channels as well as

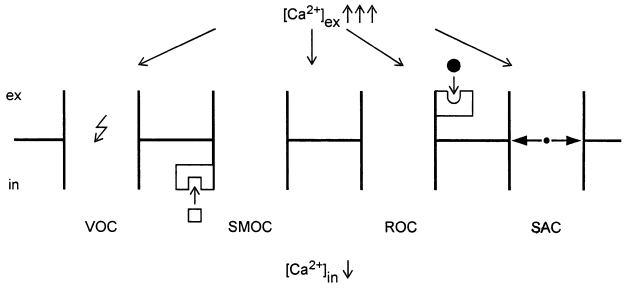

Fig. 2. Schematic diagram of various types of plasma membrane Ca^{2+} channels gating a Ca^{2+} influx down the electrochemical gradient. VOC = voltage-operated channel, SMOC = second messenger-operated channel, ROC = receptor-operated channel, SCA = stretch-activated channel.

N-type channels are activated and deactivated in a mV range closer to positive potentials than the T-type channels [99]. Since T-type channels exhibit only a short opening time, it has been proposed that they may serve as pacemakers [100]. The Ba^{2+} conductance is about 25 pS for L-type channels [101], about 9 pS for T-type channels and about 13 pS for N-type channels [102]. All types accept, besides Ca^{2+}, divalent ions such as Ba^{2+} and Sr^{2+}. Usually, Ba^{2+} and Sr^{2+} inhibit Ca^{2+} conductance. However, under certain circumstances, the conductivity of L-type channels is improved by Ba^{2+}/Sr^{2+}. The sensitivity of the voltage-dependent Ca^{2+} channels towards various pharmacological agents is markedly different. Compounds of the dihydropyridine, benzothiazepine, or phenylalkylamine group block L-type channels, most probably by switching the channels to a different gating mode [103]. Dihydropyridine increases the opening probability of L-type, but not of T- or N-type channels [100]. Cadmium blocks N- and T-type channels [104].

ω-Conotoxin, isolated from the marine snail *Conus geographus*, inhibits N-type channels and neuronal L-type Ca^{2+} channels [104], but fails to inhibit muscle L-type Ca^{2+} channels. Taicotorcin, a snake venom (*Oxyruroms S. Sutellats*) inhibits cardiac L-type Ca^{2+} channels, suggesting that different subtypes of the L-type group might exist. Physiologically, cardiac L-type channels are regulated by β-adrenoreceptor agonists via cAMP-induced phosphorylation and via stimulation by G-proteins [105]. Elevated $[Ca^{2+}]_i$ inhibits the L-type current in various tissues, whereas the T-type currents in canine ventricular and Purkinje cells are enhanced [106]. The inhibition by $[Ca^{2+}]_i$ is not observed in L-type channels reconstituted in lipid bilayers, suggesting the involvement of an cytoplasmic factor or channel/membrane/cytoskeleton interactions for the inhibitory effect [107,108]. Similar factors seem to be responsible for the instability of reconstituted L-type channels compared to T-type channels [107,109].

Muscle L-type Ca^{2+} channels consist of five subunits: an α1-subunit (155–200 kDa), an α2-subunit (130–150 kDa), a β-subunit (52–65 kDa), a δ-subunit (24–33 kDa), and a γ-subunit (30–33 kDa). The major channel-forming polypeptide is the α1-subunit, probably responsible for both ion flux and voltage sensing [110,111]. The receptors for organic Ca^{2+} channel blockers as well as the phosphorylation sites for various kinases are located on the α1-subunit. Both, α2- and γ-subunits contain membrane spanning domains, whereas δ- and β-subunits are attached extracellularly and intracellularly, respectively. The β-subunit regulates Ca^{2+} channel activity [112].

L-type and T-type Ca^{2+} channels are predominantly found in electrically active tissue. N-type channels seem to be restricted to neuronal cells, with preference to the nerve terminals, where they are involved in the negative feedback loop of neurotransmitter release [113]. Usually voltage-gated channels of different type are present in the same tissue or in the same cell [100]. Moreover, within one cell voltage-gated Ca^{2+} channels can be regionally concentrated [114].

Ligand gated Ca^{2+} channels can be subdivided into receptor-operated channels (ROC) and second messenger-operated channels (SMOC) [115]. In the ROCs receptor and ion gating subunit are part of the same molecule and binding of a ligand to the receptor on the cell surface induces a cation current (Ca^{2+} and Na^{+}) in the range of 5–50 pS [116]. ROCs are present in excitable cells, such as the glutamate-activated channel in vertebrate neurons or the ATP-activated channel in smooth cardiac and skeletal muscle cells [117–119], sensory neurons [120], macrophages [121], and exocrine cells [122].

SMOCs are gated by a messenger acting intracellularly. Activators or modulators include G-proteins, protein kinases [123–126], IP_3 or its degradation products [127,128]. The activity of the Ca^{2+} channel is closely related to the filling state of the intracellular Ca^{2+} stores [129–140]; depletion often stimulates a Ca^{2+} influx, causing the so-called capacitative Ca^{2+} influx [132–135]. The Ca^{2+} release-activated channel (CRAC) is highly selective for Ca^{2+} with a conductance of about 1 pS [136]. The mechanism how the channels are activated might involve a direct interaction of intracellular Ca^{2+} release channels with CRAC [137] or a small molecule (such as a small G-protein) released together with Ca^{2+} from the intracellular store that transduces the signal to the membrane [138–141]. Ca^{2+} entry via CRACs is further modulated by cGMP [142] and various kinases [143–145]. The state of filling of the Ca^{2+} stores thereby regulates the cGMP levels [146].

Studies on *Drosophila* photoreceptor protein which are potential candidates for capacitive Ca^{2+} entry pathways can be expected to elucidate the coupling mechanism in more detail [147–149].

Stretch-activated calcium channels (SAC) carry Ca^{2+} as well as other cations. Their open probability is also affected by the membrane potential [150]. One particular property of SACs is the sensitivity to gadolinium [151]. Usually, SACs are involved in cell volume regulation.

Within one cell, multiple pathways for Ca^{2+} entry can be present; their interrelation and regulation in concert are poorly understood [152].

2.2. Calcium decreasing mechanisms

The maintenance of Ca^{2+} gradients across the plasma membrane and the membranes of intracellular stores requires metabolic energy. The transmembrane accumulation is achieved either by *primary active* Ca^{2+}-ATPases or by *secondary active* Na^+/Ca^{2+} exchangers, which are driven by the Na^+ gradient established across the plasma membrane by the Na^+/K^+ pump and the membrane potential.

2.2.1. Calcium-ATPases

There are two different Ca^{2+} pump superfamilies, the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase family (SERCA) [153,154] and the plasma membrane Ca^{2+}-ATPase family (PMCA).

Three genes are encoding the SERCA pumps [155,156]. Because of alternative splicing, both SERCA 1 and 2 have two isoforms. The isoforms differ in their tissue distribution. SERCA 1 is found in fast twitch skeletal muscle and the expression of SERCA 1a and 1b is developmentally regulated [155,157]. SERCA 2a is expressed mainly in heart and slow twitch skeletal muscle, SERCA 2b is found in nearly every tissue. Large and small intestine, spleen and lung are the preferential tissues for SERCA 3. It is also found in brain, stomach, uterus, skeletal muscle, and heart [156]. The expression of SERCA 1 is controlled by hormones and growth factors [158]. The resulting five different isoforms have molecular weights varying from 100–115 kDa. The homology of the isoforms varies between 75 and 85%. The arrangement in the plasma membrane and the domains for putative Ca^{2+} binding are highly conserved among the isoforms [155]. There seem to be 10 (11 for SERCA 2b) transmembrane domains, a small segment inside the organelle and a big cytosolic part containing the calcium binding domain, the ATP binding domain, and the phosphorylation site during Ca^{2+} binding [159]. The Ca^{2+} binding site differs from the well-known EF-hand in the Ca^{2+} binding protein. Transmembrane helices 4, 5, 6, and 8 appear to be involved in Ca^{2+} binding [160].

Two moles of Ca^{2+} ions are transported per mole ATP. The affinity constant varies between 0.4 and 1.1 μM and the maximum velocity between 0.5 and 5 μmol/mg protein/min [161,162]. The affinity for Ca^{2+} can be greatly enhanced by phospholamban. This homopentameric molecule, consisting of subunits with a molecular weight of 6 kDa, regulates SERCA 1 and 2. Upon phosphorylation, the inhibitory action of phospholamban on the pump is abolished and the Ca^{2+} affinity of the pump is increased. Thapsigargin, a tumor-promoting sesquiterpene lactone, is a highly effective and specific inhibitor of the SERCAs [163,164], whereas heavy metals like Cd^{2+} inhibit Ca^{2+} pumps both in the plasma membrane and intracellular stores [165,166].

Four genes have been demonstrated to encode the plasma membrane Ca^{2+}-ATPase (PMCA). There exist several isoforms due to alternate splicing [167]. The molecular weights range from 127–136 kDa. The expression of the PMCAs in various tissues is quite different (Table 1). PMCA 1 transcripts have been detected in nearly all tissues, whereas the expression of PMCA 2–4 mRNA is highest in brain [168].

The PMCAs belong to the P-type ion pumps with a stoichiometry of 1 mole Ca^{2+} transported per mole ATP split [152]. There is evidence for 10 transmembrane domains connected by small extracellular loops and large intracellular loops that represent nearly 80% of the pump mass. The latter contain the ATP binding site, the aspartate phosphate binding site, the calmodulin binding domains, and the phosphorylation sites for protein kinases A and C [169,170]. The binding of calmodulin results mainly in an increase of the Ca^{2+} affinity [171] but there is also evidence for an inhibitory effect of calmodulin binding due to spatial obstruction of the Ca^{2+} binding site [172]. The Ca^{2+} affinity of the pump increases 40-fold upon calmodulin activation [173]. PMCA are also directly

activated by stimulating agonists [174]. In vitro, the Ca^{2+}-activated intracellular protease calpain can stimulate the pump by limited proteolysis [175,176]. In vivo, the situation remains to be elucidated since calmodulin can slow down proteolysis and change the sites of protein cleavage [176].

2.2.2. Sodium / calcium exchanger

The Na^+/Ca^{2+} exchanger has been found in a number of cells and tissues [177–181]. Several isoforms of the classical Na^+/Ca^{2+} exchanger have been cloned from at least three different genes (NCX 1–3) [182,183].

The exchanger from canine cardiac SR is a polypeptide with a molecular weight of about 110 kDa [184,185]. The proposed topology of the Na^+/Ca^{2+} exchanger consists of 11 transmembrane segments. An intracellular loop between segments 5 and 6 seems of particular importance. It contains an inhibitory region and a regulatory site with a high Ca^{2+} affinity. The former is involved in the Na^+-dependent inactivation of the exchanger [183,186].

The exchanger has a stoichiometry of 3 Na^+:1 Ca^{2+} [187]. Its function is dependent on an intact actin cytoskeleton [188]. In addition to Na^+ and Ca^{2+}, the activity of the antiporter is modulated by ATP [189], Mg^{2+} [190,191], and hydrogen ions [192–194]. The effect of protons is asymmetric—inhibition at the cytoplasmic side of the transporter and stimulation at the periplasmic side [195]. Moreover, part of the inhibition requires the presence of Na^+. The functional consequences of this pH sensitivity are not yet clear.

The Na^+/Ca^{2+} exchanger can operate in reverse [196,197]. This reversed mode may have physiological implications, e.g., for heart muscle contraction, in that it can trigger Ca^{2+} release from SR in parallel to the voltage-dependent Ca^{2+} channels [198–200].

The relative importance of the Na^+/Ca^{2+} exchanger in adjusting and maintaining cellular Ca^{2+} levels varies from cell to cell. In cells that undergo massive intracellular Ca^{2+} changes, like heart cells, the high capacity Na^+/Ca^{2+} exchanger plays the dominant role. When a fine adjustment at low Ca^{2+} levels is necessary, the Ca^{2+}-ATPases seem to be more important [201–203].

2.3. Calcium buffers

The role of intracellular Ca^{2+} buffers in determining $[Ca^{2+}]_i$ are often neglected. Their importance becomes evident by performing a short calculation. Basal $[Ca^{2+}]_i$ is between 50–200 nM, increasing to around 1–1.5 μM after hormonal stimulation. However, from a total cellular Ca^{2+} content of around 12 nmol/mg protein around 25% can be released by hormones, which should result in an increase in $[Ca^{2+}]_i$ to 0.5 to 1 mM [28]. Thus, during stimulation only a small portion of the released Ca^{2+} remains as free Ca^{2+}, most is bound to Ca^{2+} buffers. In chromaffin cells Neher and Augustine [204] determined that 98–99% of the Ca^{2+} entering the cell is taken up by endogenous Ca^{2+} buffers.

Calcium buffers can be subdivided into molecular buffers, represented by the cytosolic CaP and organelle buffers, mainly represented by mitochondria and ER. CaP have high or low affinity binding sites. CaP with high affinity binding sites are present in low concentrations (low capacity), whereas intracellular concentrations of low affinity CaP are much higher (high capacity). Ca^{2+} binding to high affinity binding sites often induces changes of the protein structure, thereby evoking certain cellular responses [27,205].

Within the cell there are inorganic, small organic, and macromolecular Ca^{2+} ligands. Aside from their role in the formation of the skeletal structure, Ca^{2+} salts play a role under pathological conditions, when inflammation-induced changes in the microenvironment, like pH, ionic strength lead to a formation of microprecipitates and stones [206]. L-glutamate, citrate, and ATP are examples of small organic Ca^{2+} ligands; macromolecular ligands are calmodulin, calsequestrin, etc. Total Ca^{2+} buffer capacity has been determined in human neutrophils to be around 0.76 mM with an apparent single dissociation constant of 0.55 μM [207].

In Table 2, Ca^{2+} buffering molecules and their Ca^{2+} affinity are compiled. The CaP parvalbumin, a muscle protein from marine organisms, has been studied extensively. These molecules can bind up to four Ca^{2+} ions, like troponin and calmodulin [217,209]. A Ca^{2+} binding domain consists of an amino acid loop together with two helices, the so-called EF-hand. In Ca^{2+} binding, the conformation of

Table 2
Intracellular Ca^{2+} ligands

Ligand	pK_d	Approx. mol. wt.	Refs
Citrate	3.5	249	[208]
Glutamate	1.4	144	[208]
ATP^{4-}	4.1	502	[208]
Calmodulin	5.5–6.7	16,700	[208]
Troponin C	5–7	18,000	[209,210]
Intestinal Ca^{2+} binding protein	6.5	88,000	[211]
Calpactin I	4–5.3	49,000	[212]
Calpactin II	5	39,000	[212]
Calelectrin	4–5	35,000–67,000	[213]
Protein II	4.6	32,500	[214]
Synexin	3.7	47,000	[215]
Gelsolin	2–2.7		[216]

the protein changes, exposing a hydrophobic region, enables the protein to interact with Ca^{2+} pumps, ion channels [218], the cytoskeleton, or a variety of kinases [219] and phosphatases [220]. Molecules containing such Ca^{2+} binding domains, like the intestinal CaP [221] or the S-100 protein belong to the 'calmodulin superfamily' of CaPs. Their Ca^{2+} affinities are close to $[Ca^{2+}]_i$ levels during cellular stimulation [222].

A second class of CaPs (Calpactin I and II, protein II, calelectrin, and synexin I—all categorized into the annexin group) show a calcium-dependent reversible binding to membranous or cytoskeletal structures [223]. Their Ca^{2+} affinity is low, with a K_D of about 10 to 100 μM [224]. However, in the presence of specific phospholipids, the Ca^{2+} affinity is greatly increased and Ca^{2+} ions bind in a cooperative manner [214,225]. Their physiological role may be to regulate cell growth [226] as well as membrane fusion [227].

The characteristic structure of this second class of CaP is a 70–72 amino acid chain, which can be repeated up to four times and shows a 30% similarity with a few invariant residues [228]. The structure of the Ca^{2+} binding domain seems to be distinct from the EF-hand of calmodulins.

The contributions of different Ca^{2+} ligands to cellular Ca^{2+} buffering can be estimated in comparing citrate and calmodulin. The intracellular concentration of the low affinity Ca^{2+} buffer citrate is approximately 0.5 mM compared to the concentration of 20 μM (functional binding capacity 80 μM) of the high affinity Ca^{2+} buffer calmodulin. Under resting conditions, at 0.1 μM $[Ca^{2+}]_i$, calmodulin can bind around 200 times more Ca^{2+} than citrate and 80 times the amount of free Ca^{2+}. With increasing $[Ca^{2+}]_i$ the ratio changes: at 5 μM Ca^{2+} 80 times more Ca^{2+} is bound to calmodulin than to citrate [208]. The high affinity buffers operate most effectively in a range of 1–10 μM $[Ca^{2+}]_i$, values slightly above the upper range of concentrations reported for hormone-induced Ca^{2+} peaks. The upper limit of 10 μM might be important in certain cellular regions, e.g., subplasmalemmal areas, where extreme local changes have been observed [229].

Restriction of a Ca^{2+} signal to this range is of vital interest to the cell. Elevation above this level can lead to cellular destruction, e.g., by activation of calpains, Ca^{2+}-dependent cysteine proteases, with a K_D for Ca^{2+} of about 30 μM for type I and 500 μM for type II calpain [230,231].

Finally, a few comments on intracellular organelles and their role as Ca^{2+} buffers. We will focus here on mitochondria since ER has been discussed before. Different intramitochondrial pools exist that can be distinguished on the basis of their sensitivity to the metabolic inhibitors, FCCP and CN [232]. These pools are involved in buffering of an extramitochondrial Ca^{2+} load and it can be calculated that around 30% of the total cellular Ca^{2+} load can be buffered by mitochondria [233]. Recent results, however, using aequorin targeted to mitochondria, suggest a rather minor role for mitochondria in Ca^{2+} buffering [234].

Ca^{2+} uptake into mitochondria is mediated by a uniporter driven by the electrochemical gradient. The Ca^{2+} transport capacity is high and can be inhibited by ruthenium red and lanthanides. Calcium efflux is mediated by two different pathways, a Na^+-dependent pathway, most probably a Na^+/Ca^{2+} exchanger, and a Na^+-independent pathway [235,236].

2.4. Calcium signalling pattern

Electrical and humoral stimuli can evoke a number of different responses of intracellular Ca^{2+} depending on the relative activities of Ca^{2+}-increasing and decreasing mechanisms as well as extracellular factors like type and concentration of stimulus [46]. The

changes in intracellular Ca^{2+} may be uniform through the whole cell or may be restricted to certain areas. Furthermore, the changes can vary throughout stimulation, resulting in spatial and temporal signalling patterns.

The simplest response is a uniform Ca^{2+} increase within the whole cell, usually as a 'peak and plateau' sequence. In pancreatic acinar cells, as in many other cells, this sequence can be initiated by supramaximal doses of agonists (Fig. 3) [237]. Receptor occupation and G-protein-linked activation of phospholipase C result in the formation of IP_3, which releases Ca^{2+} from intracellular stores (= peak phase), followed by a sustained influx of extracellular Ca^{2+} across the plasma membrane (= plateau phase) [238]. Store depletion seems to trigger the sustained Ca^{2+} influx either by a kind of diffusible messenger, which seems to be phosphatase sensitive [139,239], or by a particular arrangement of plasma membrane channels and intracellular Ca^{2+} stores. After termination of stimulation, intracellular Ca^{2+} release and transmembrane influx cease and Ca^{2+} is sequestered back into the intracellular stores or extruded out of the cell. However, there exist ample modifications; e.g., in muscle cells Ca^{2+} pools of SR are depleted by the interaction of the voltage sensor of the dihydropyridine receptor and the intracellular ryanodine channel [240]. Likewise, in several cases intracellular stores are not involved in the Ca^{2+} response and changes of intracellular Ca^{2+} are solely induced by movements of Ca^{2+} across the plasma membrane [241].

Spatial Ca^{2+} responses are manifested as Ca^{2+} waves. They have been described first in eggs upon fertilization. Ca^{2+} waves have also been found in heart muscle cells, pancreatic acinar cells, hepatocytes, and endothelial cells. There are slow and fast Ca^{2+} waves with propagation rates ranging from $\mu m/s$ to 100 $\mu m/s$ [242]. Fast waves usually cross the whole cell, whereas slow waves propagate within the subplasmalemmal area. Another distinction is based on the steepness of the Ca^{2+} change at the

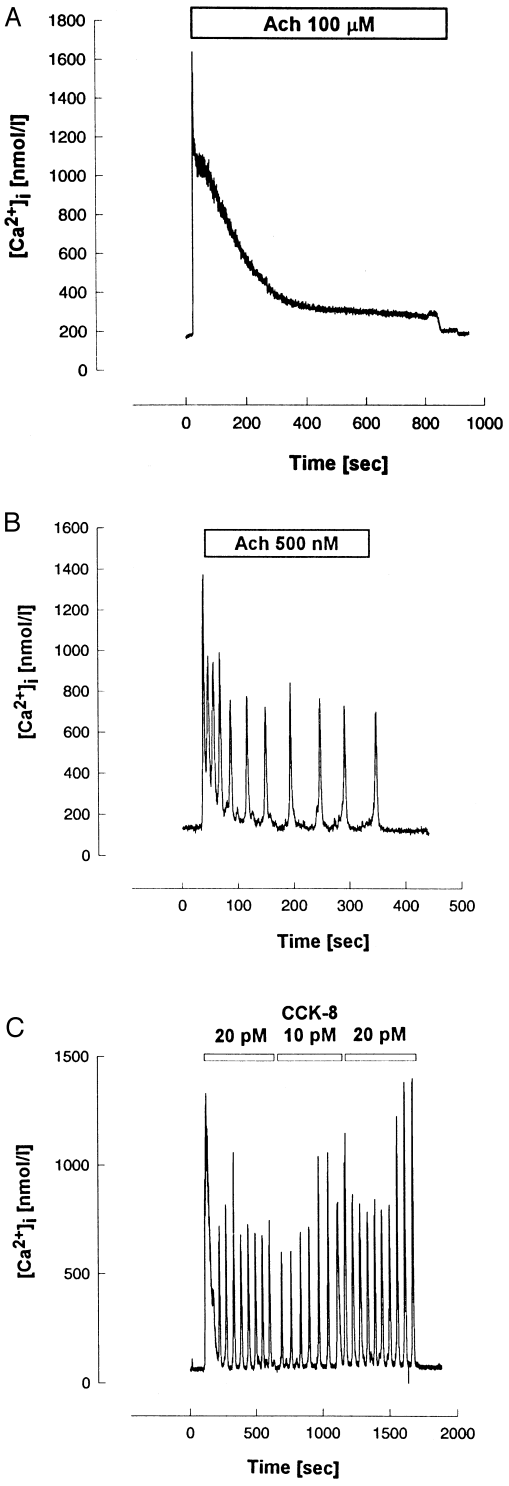

Fig. 3. Effect of different agonists and concentrations on intracellular Ca^{2+} signals in single pancreatic acinar cells. A supramaximal concentration of acetylcholine (ACH, 100 μM) results in a peak plateau sequence of intracellular Ca^{2+} concentration (A). A physiological concentration of ACH (500 nM) results in cytosolic Ca^{2+} oscillations (B). The frequency of oscillations can be modulated by different concentration of secretagogues, e.g., cholecystokinin (C).

wave front. In cardiac cells, a sudden sharp increase of Ca^{2+} concentration at the wavefront has been demonstrated, whereas in hepatocytes, Ca^{2+} concentration rises progressively in the direction of wave propagation [243]. Moreover, when Ca^{2+} wave propagation occurs, spiral Ca^{2+} waves can be induced along the wave front [244]. Several models have been developed to explain these observations. The basis of Ca^{2+} wave propagation is similar to a reaction–diffusion mechanism [245–247].

Ca^{2+} waves are frequently associated with Ca^{2+} oscillations. Ca^{2+} oscillations are highly variable in frequency, amplitude, and shape of the Ca^{2+} spikes potentially coding for huge amounts of information [248]. This variability depends on cell types and kind and concentration of the stimulant (Fig. 3) [237,249–251]. Within one cell type the same agonist can evoke different oscillation patterns [252]. Several different models have been proposed to explain the occurrence of Ca^{2+} oscillations [253–255]. It has been suggested that Ca^{2+} oscillations are caused by periodic changes in IP_3 concentrations via feedback by Ca^{2+} and PKC. However, alternating IP_3 concentrations have not been demonstrated so far, instead, constant IP_3 concentrations using a non-metabolizable IP_3 analog have been shown to evoke Ca^{2+} oscillations [256]. Like in Ca^{2+} waves, an important underlying principle of Ca^{2+} oscillations seems to be calcium-induced calcium release [257]. An initial Ca^{2+} release provokes a further Ca^{2+} increase; however, above a certain concentration a negative feedback occurs. Because of the bell-shaped Ca^{2+} dependency of its activity, the intracellular Ca^{2+} release channel closes and Ca^{2+} is sequestered until the next cycle starts. The inhibitory action of Ca^{2+} alone does not seem to be sufficient for explanation. There is evidence for stores of different IP_3 sensitivity or IP_3 receptor density in pancreatic acinar cells [45,258] which may be the cause for the spatially restricted Ca^{2+} signals. Recently, Thorn et al. [259] demonstrated that a localized Ca^{2+} spike is the result of the coordinated action of multiple single Ca^{2+} release events. Localized 'hot spots' resembling the local puffs shown in Xenopus oocytes [260] initiate Ca^{2+} release from surrounding regions. Moreover, the repetitive temporal Ca^{2+} signalling patterns as well as the Ca^{2+} waves are influenced by the activity of the Ca^{2+} sequestering pumps [261,262] and the extent of transmembrane Ca^{2+} movement [263]. Finally, Ca^{2+} oscillations are also modulated by intercellular communication via gap junctions [264,265].

3. Calcium homeostasis and calcium signalling in disease states

During recent years the role of Ca^{2+} in the development and maintenance of disease states has been demonstrated in a number of instances. While its role has long been implicated in cardiovascular diseases, there is now increasing evidence for Ca^{2+} being involved also in other pathophysiological processes, such as inflammation, stroke, diabetes, and tumor spreading. Within the scope of this review it is not possible to discuss all these cases in detail. Therefore, the authors will concentrate on four different examples of disturbed cellular Ca^{2+} homeostasis: inflammatory processes, heart diseases, hypertension, and neurologic disorders.

3.1. Inflammatory processes

Inflammation is a process in which several cell types are involved. In a simplified model, a physical or chemical factor damages cells of a certain organ or tissue. This damage activates the immune system represented by granulocytes and lymphocytes. The tissue damage plus the immune response yields the inflammatory process which may be restricted to an area or organ or may have systemic effects like in sepsis. There are several examples that disturbances of $[Ca^{2+}]_i$ represent an early feature of inflammation and may have consequences on maintenance of pathological state. In the following, the role of Ca^{2+} in the impairment of cellular integrity by physical, chemical, or biological irritation will be highlighted first. Then, the Ca^{2+}-dependent responses of the immune system will be discussed.

3.1.1. Tissue damage

Any agent that is able to disrupt plasma membrane integrity will cause an increase in $[Ca^{2+}]_i$. This is the case for chemical irritation of cells, e.g., by certain antibiotics like the Ca^{2+} ionophores. However, there

are more specific toxins. The B-subunit of the cholera toxin evokes an increase of $[Ca^{2+}]_i$ in lymphocytes without affecting IP_3 levels or the PKC pathway [266]. *Clostridium difficile* toxin A application results in an increase in $[Ca^{2+}]_i$ in neutrophils [267], followed by a disassembly of cytoskeleton [268]. Hemolysin producing *Escherichia coli* strains induce a massive Ca^{2+} increase in human platelets [269] and a number of hepatoxins, like bromobenzene or acetaminophen, disrupt cellular Ca^{2+} homeostasis [270,271], thereby causing blebbing of plasma membrane [272]. One reason is the inhibition of the plasma membrane Ca^{2+} pump, causing saturation of intracellular Ca^{2+} buffers, followed by an increase in $[Ca^{2+}]_i$ and depletion of intracellular ATP associated with a derangement of the cellular cytoskeleton [273,274]. Most likely, this mechanism is involved also in heavy metal cytotoxicity [275].

For the CCl_4-induced liver cell necrosis, a role of $[Ca^{2+}]_i$ has also been demonstrated. CCl_4 initially impairs the integrity of a variety of Ca^{2+} stores and Ca^{2+} buffer organelles. Later, the plasma membrane becomes leaky, permitting an additional massive Ca^{2+} influx, a process in which activation of phospholipase A2 seems to be involved [276]. Likewise Hg^{2+}, besides inhibiting the plasma membrane Ca^{2+} pump, releases Ca^{2+} from intracellular stores that are distinct from thapsigargin-sensitive stores [277].

In a duct ligation-induced pancreatitis [278] in rats, it could be demonstrated that increases in basal $[Ca^{2+}]_i$ represents an early sign of acute pancreatitis [279]. Furthermore, Ca^{2+}-dependent hormone and secretory functions are impaired. The same phenomenon is observed in the cerulein hyperstimulation model of acute pancreatitis [280] where the characteristic spatial Ca^{2+} signalling pattern during hormone stimulation is lost [281]. Interestingly, the capacity of Ca^{2+} stores in normal and inflamed cells is the same, suggesting a defect at the level of receptor/G-protein interaction or the IP_3 channels.

Inflammation also affects the main cellular redox system, glutathione (GSH) [282]. Ca^{2+} and GSH levels influence each other. Increased $[Ca^{2+}]_i$ lowers the cellular GSH content [283]; decreased GSH levels increase Ca^{2+} entry into the cell [270]. On the other hand, exogenous GSH inhibits the Ca^{2+} ionophore-mediated Ca^{2+} entry or thapsigargin-evoked Ca^{2+} influx in gastric mucosal cells [284]. In human T-cells the thapsigargin-induced Ca^{2+} influx is, however, not affected by GSH levels [285]. Instead, Ca^{2+} influx stimulated via the T-cell receptor decreases with decreasing GSH concentration. Such mechanism might act as a switch between an antigenic mode—resulting in cell proliferation—and an inflammatory mode—resulting in secretion of inflammatory mediators. Thereby, GSH probably modifies the signal transduction pathway at the level of the tyrosine kinase.

3.1.2. Immune response

In order to orchestrate the different cell populations involved in inflammation—epithelial and endothelial cells, macrophages, neutrophils, eosinophils, basophils, and lymphocytes—a number of biologically highly potent mediators like cytokines and arachidonic acid metabolites are utilized. Ca^{2+} is involved in the stimulus-induced secretion of several mediators. Lipopolysaccharide (LPS)-induced secretion of interleukin (IL)-1α, IL-1β and IL-10 is affected by Ca^{2+} and calmodulin inhibitors in murine peritoneal macrophages. Both LPS and Anti-IgE-evoked IL-6 production in peritoneal mast cells are dependent on the presence of extracellular Ca^{2+} [286]. IL-4 transcription of the CD4+ helper cell subset Th2 depends solely on the presence of Ca^{2+} [287]. Interestingly, gene expression of T-lymphocytes varies not with the way by which changes in $[Ca^{2+}]_i$ are evoked [288]. But a $[Ca^{2+}]_i$ increase per se does not seem to be sufficient for cytokine release in every case [289,290] since the T-lymphocyte synthesis and secretion of interleukin-2 is only partially controlled by a continuous Ca^{2+} influx [131,291]. The formation of leukotrienes in mast cells, eosinophils and basophils [292] as well as in epithelial cells like gastric chief cells [293] through the 5-lipoxygenase pathway is promoted by extracellular Ca^{2+}. This might have important implications for asthmatic and other allergic diseases where an eosinophilic hyperactivity is thought to be the underlying mechanism.

In neutrophils of patients with Crohn's disease, the leukotriene-induced changes in $[Ca^{2+}]_i$ are impaired [294]. Moreover, impaired Ca^{2+} signalling in leukocytes is a common feature of chronic inflammatory diseases. For example, in active rheumatoid arthrosis, changes in $[Ca^{2+}]_i$ evoked via the Fc receptors are depressed [295], whereas in the quiescent phase of the disease, no defect in Ca^{2+} signalling is observed.

Likewise, phytohemagglutinin or anti-CD3-induced changes in $[Ca^{2+}]_i$ of T-lymphocytes are affected in patients with inflammatory bowel disease and rheumatoid arthritis [296].

The systemic inflammatory mediator tumor necrosis factor α is able to impair both resting and hormone-stimulated Ca^{2+} levels in cell culture [297], whereas other cytokines are able to enhance mobilization of Ca^{2+} from intracellular stores [298].

Cytokines and related substances evoke many of their effects via changes in $[Ca^{2+}]_i$ [292]. The IL-3-promoted adhesion of human basophils to endothelia is improved by extracellular Ca^{2+} [299]. Moreover, chemokines induce leukocyte migration by increases in $[Ca^{2+}]_i$ [300,301]. In addition, the permeability changes of endothelial cells during leukocyte evasion are promoted by intra and extracellular Ca^{2+}, probably mediated via integrins and cytokines, in particular IL-1 [302,303]. IL-1 has been shown to increase $[Ca^{2+}]_i$ by stimulating Ca^{2+} influx across the plasma membrane. This effect depends on an intact arrangement of cell–cell and IL-1 receptor–cytoskeletal interactions [304]. Tight junctions permeability is also increased by mobilization of Ca^{2+} from intracellular stores [305].

Killer cells increase $[Ca^{2+}]_i$ in their target cells markedly, which induces necrosis or apoptosis [306,307]. Interestingly, the whole process can be prevented by increasing the intracellular Ca^{2+} buffer capacity. Similar mechanisms are responsible for the cytotoxic activity of the tumor necrosis factor α [308]. Although all of these responses should be helpful in overcoming inflammation, an excessive activation seems to be deleterious and may even maintain the inflammatory state. This might explain the beneficial effect of organic Ca^{2+} channel blockers in suppressing the inflammatory response [309].

3.2. Heart failure

In heart muscle an excessive increase in $[Ca^{2+}]_i$, 'calcium overload' is responsible for lesions of myofibrils and mitochondria, and finally for cell death [310].

The most important cause for 'calcium overload' is an increased permeability of the plasma membrane for calcium, most frequently induced by perfusion reduction [311].

Cellular calcium changes during ischemia occur in two different phases. Initially, the cellular ATP stores are depleted since mitochondrial respiration is inhibited. In parallel, the collapse of the H^+ gradient across the inner membrane of the mitochondria leads to a release of mitochondrial Ca^{2+}. The increase in $[Ca^{2+}]_i$, in turn, activates Ca^{2+}-stimulated ATPases reducing ATP content further. In addition, proteases that degrade cellular structures are activated [312]. At this early stage the disintegration of the membrane barrier has no severe consequences since due to the reduced perfusion of the extracellular space, the Ca^{2+} gradient across the plasma membrane diminishes. Furthermore, ischemia-induced tissue acidosis decreases Ca^{2+} entry through voltage-dependent Ca^{2+} channels [313]. In the second phase, when reperfusion of the ischemic area starts, the Ca^{2+} gradient across the plasma membrane is re-established. Ca^{2+} flows into the cells and the Ca^{2+} extrusion capacity of the cells is exceeded [314]. Moreover, the cytokine IL-1 impairs the protective effects of protons on Ca^{2+} currents during acidosis [315]. The extrusion capacity is also decreased because the reduced Na^+ gradient lowers the Na^+/Ca^{2+} exchanger activity [316]. Moreover, a reversal of the Na^+/Ca^{2+} exchanger might increase $[Ca^{2+}]_i$, perhaps even activated by oxygen-derived free radicals [317]. Finally, the Ca^{2+} increase results in cellular necrosis probably by activating a number of degrading enzymes like ribonucleases and proteases. This model has been confirmed by observations that the extent of cell necrosis is markedly reduced when the massive Ca^{2+} influx is inhibited by application of Ca^{2+} channel blockers [318].

A decrease in $[Ca^{2+}]_i$ buffer capacity can also amplify the effect of the massive Ca^{2+} influx since application of the Ca^{2+} chelator BAPTA, which increases intracellular Ca^{2+} buffer capacity, reduces post-ischemic ventricular fibrillations in dogs [319]. The reduction of ATP and the modification of other Ca^{2+} buffering molecules might be important factors.

In heart muscle cells of patients with end-state heart failure, a reduced availability of Ca^{2+} has been reported. Especially, Ca^{2+} release and Ca^{2+} re-uptake from intracellular stores seem to be impaired [320,321]. Cytosolic factors [322,323] as well as decreased mRNA levels for the SR Ca^{2+} release channel (ryanodine receptor), phospholamban, and for the

Ca^{2+}-ATPase isoform have been implied [324–326]. Additional post-translational regulation cannot be excluded. Expression of ryanodine receptors was also decreased significantly in patients with ischemic cardiomyopathy, whereas the opposite was observed in a group with idiopathic dilated cardiomyopathy [326]. These findings suggest that intracellular Ca^{2+} homeostasis is impaired in dilated cardiomyopathy. A reduced refilling of intracellular Ca^{2+} stores during diastole decreases the amount of Ca^{2+} available for the next contraction.

In end-stage heart failure, an increase of mRNA and protein levels of the Na^+/Ca^{2+} exchanger has been observed [327]. The enhanced expression of the exchanger may have consequences for the electrical stability of heart tissue because late depolarizations due to the electrogenic exchanger operation can occur. These may cause the well-known arrythmias in patients with chronic heart failure.

The mechanisms leading to different expression patterns of Ca^{2+}-transporting proteins are poorly understood. From developmental studies on muscle cells, a control of Ca^{2+} transport proteins by mitogens and growth factors is well known [328,329]. Calcium oscillations in cultured rat myocytes, which depend on the presence of a ryanodine-sensitive Ca^{2+} release, rely on the presence of tumor growth factor $\beta 1$ [330]. Recently, interleukin 1β has been shown to downregulate Ca^{2+}-regulating genes in cultured rat ventricular myocytes [331]. Changes of mRNA and protein levels during cardiac diseases might be induced by similar mechanisms.

3.3. Hypertension

An important pathophysiological parameter in hypertension is the increased availability of intracellular Ca^{2+} [332]. This increase forms the basis for the therapeutic use of Ca^{2+} channel blockers like nifedipine, etc. [111]. The Ca^{2+} channel blockers reduce intracellular Ca^{2+} availability and thereby impair excitation–contraction coupling in vascular smooth muscle cells, the major determinant of vascular tone [333].

Although a few studies reported unchanged or decreasing values of $[Ca^{2+}]_i$ during hypertension [334,335] the increase in $[Ca^{2+}]_i$ seems to be established as the major pathophysiological factor. An increase in $[Ca^{2+}]_i$ has been described in platelets, erythrocytes, lymphocytes, aorta, and in renal cells either in patients with hypertension or in spontaneously hypertensive rats [336–338]. The increased level of free Ca^{2+} can be the result of either an increased Ca^{2+} influx or a decreased Ca^{2+} extrusion.

Several causes for the disturbance of $[Ca^{2+}]_i$ have been proposed. It has been known for a long time that hypertension is associated with decreased serum Ca^{2+} and a regulatory increase in parathyroid hormone levels in extreme cases with consequences for bone mineralization [339]. Renal loss of Ca^{2+} [340–343] and a decreased intestinal uptake have been observed [344,345]. A high Ca^{2+} diet often normalizes the vascular tone especially under conditions of an enhanced need for Ca^{2+}, like in pregnancy [346,347]. Moreover, the relationship between intra and extracellular Ca^{2+} concentration is normalized. As an underlying mechanism [348–350], an alteration in activity or amount of calmodulin has been postulated [351]. In a number of animal studies, a loss either in activity or amount of calmodulin has been described in several tissues like heart, aorta, brain, or duodenum [352–355] and a recovery of calmodulin activity in spontaneously hypertensive rats has been shown after a high Ca^{2+} diet [353]. However, in other models an increase in calmodulin-dependent cellular function, such as muscle contractions or the increased activity of the Na^+/H^+ antiporter have been observed in hypertension [356,357].

The imbalance of Na^+ homeostasis in primary hypertension is well established [358–360]. Enhanced Na^+ uptake often combined with renal Na^+ retention represents an important risk factor [361–363]. In parallel to Na^+ retention, intracellular Na^+ increases, which is accompanied by an increase in $[Ca^{2+}]_i$, probably by affecting the Ca^{2+} extrusion systems [364].

The same Ca^{2+}-ATPase activity has been found in erythrocytes from patients with primary hypertension and in normotensive controls [365], thus the Na^+/Ca^{2+} exchanger is most probably responsible for the increase in $[Ca^{2+}]_i$. Increasing $[Na^+]_i$ reduces the driving forces for the antiporter, thereby partially inhibiting the high capacity pathway of Ca^{2+} extrusion. The Ca^{2+}-ATPase is able to compensate for the loss of activity of the antiporter only under resting conditions and at the expense of the loading of

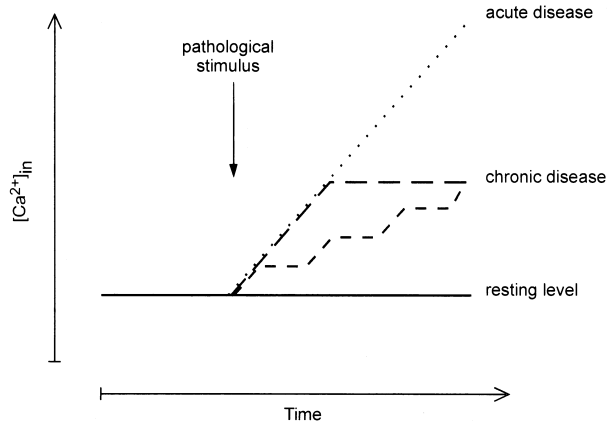

Fig. 4. The extent of alterations of resting cellular Ca^{2+} homeostasis after a pathological stimulus predicts the cellular outcome.

intracellular stores [366] (Fig. 4). Conversely, after stimulation, Ca^{2+} transients are markedly enhanced and induce increased cellular responses [367]. Such exaggerated Ca^{2+} responsiveness is probably not restricted to vascular smooth muscle cells, an enhanced release of catecholamines from sympathetic neurons or a suppressed production of the endothelial derived relaxing factor nitric oxide can also contribute to the enhanced vascular tone [368].

The increase in $[Na^+]_i$ is probably due to multiple reasons. One involves the Na^+/H^+ exchanger (NHE) that participates in regulation of intracellular pH, of cell volume, and of cell growth [369]. At least in phenotypic subgroups of patients with hypertension, the transport activity of the NHE in blood cells as well as in smooth muscle cells is increased [370,371]. This abnormality seems to be genetically fixed [372] but no changes in the cDNA sequence could be observed [370]. On the other hand, higher Ca^{2+} levels per se stimulate the NHE activity [356,357]. Recently, an enhanced pertussis toxin-sensitive G-protein activity has been found in immortalized cell lines from hypertensive patients [373], suggesting a change in the signal transduction cascade with potentially stimulating effects on NHE activity and Ca^{2+} signalling.

Another explanation for the increase in $[Na^+]_i$ is that in nearly 50% of patients with hypertension, increased levels of 'endogenous ouabain' have been found [374] that inhibits the Na^+,K^+-ATPase [375–377]. In addition, chronic administration of ouabain is able to induce hypertension in animal studies [378,379], whereas short-term incubation has no effect on resting Ca^{2+} levels in isolated cells [380].

Taken together, the chronic cellular Ca^{2+} overload due to increased Na^+ levels and perhaps, altered levels of intracellular CaPs like calmodulin, induce higher basal activity and hyper-responsiveness in vascular smooth muscle cells and blood pressure regulating endothelial or ganglion cells with a consecutive rise in systemic blood pressure.

3.4. Neuronal damage

In no other tissue is the role of Ca^{2+} as multifunctional as in the brain. In neuronal cells there are various types of voltage-gated channels (L-, T-, N-, P-type channels) as well as the ligand operated channels; the latter being primarily gated by glutamate. In addition, there are second messenger operated channels which are activated on depletion of intracellular Ca^{2+} stores. Calcium is extruded from the cells by the activity of active Ca^{2+} pumps and by a Na^+/Ca^{2+} exchanger [381]. Besides its role as a regulator of metabolic pathways and as a link between stimulus and secretion, Ca^{2+} in brain is proposed to be involved in memory formation and cognitive functions [382]. Within this chapter, we would like to focus on ischemic and neurodegenerative disorders.

Occlusion or rupture of a brain tissue supplying blood vessel results in decreased perfusion with hypoxia and ischemia. Patients show neurologic dysfunctions ranging from paralysis of a single group of muscles to complete unconsciousness. In the steps leading from ischemia to neuronal cell death, Ca^{2+} ions plays an important integral part, summarized as the 'exitoxicity concept' [383–385].

Hypoxia-induced cellular ATP depletion causes cell depolarization resulting in the release of neurotransmitters, in particular the excitatory amino acid glutamate, that accumulate in the extracellular space. Glutamate stimulates neural cells via the kainate/quisqualate (K/Q) and the *N*-methyl-D-aspartate (NMDA) channel located on the postsynaptic membrane. The former is permeable solely to monovalent cations whereas the latter also transports Ca^{2+} with a high conductance. The effects of glutamate on cellular Ca^{2+} signals are not restricted to the time period when the neurotransmitter is pre-

sent. Very short repetitive pulses of glutamate (1–3 s) can evoke extremely long-lasting Ca^{2+} increases, stable for around 20 min, which depend on extracellular Ca^{2+} [386]. Prolonged exposure (15 min) of cultured neuronal cells to glutamate results in cell death which starts about 2 h after termination of glutamate application and also requires the presence of extracellular Ca^{2+} [387]. Furthermore, protein kinase C activation prolongs cation influx [388].

The relevance of these in vitro data for the situation in vivo has been demonstrated using antisense DNA to NMDA-R1 receptor channels in rats; their intravenous application reduced focal ischemic infarctions [389].

Application of NMDA antagonist fails to have neuroprotective effects in global ischemia [390]; therefore, voltage-activated Ca^{2+} channels and the Na^+/Ca^{2+} antiporter are likely candidates for the additional Ca^{2+} influx [391]. The increased Ca^{2+} influx might be balanced by the cells as long as there is sufficient metabolic energy available, as for example in a perifocal area of an infarct. In such a zone, application of NMDA antagonists should prevent further increases in intracellular Ca^{2+} since the cell still has Ca^{2+} extrusion capacity. In contrast, when cells are totally depleted of ATP, e.g., in the central zone of an infarction area, the cells are flooded with Ca^{2+}. The potential therapeutic strategy should, therefore, involve at least three different tools: drugs to block the nonselective cation channels, dihydropyridine receptor antagonists, and NMDA receptor antagonists [382]. Recently, a novel mechanism responsible for the increased Ca^{2+} permeability has been described. It involves an altered phosphoinositol cascade. Especially IP_4 has been shown to accelerate Ca^{2+} entry in CA1 neurons after ischemia [392,393].

Also, changes in functional Ca^{2+} buffer capacity have been shown to be of importance in neurological diseases. In rat hippocampal slices for example, cell death can be prevented by increasing the intracellular Ca^{2+} buffer capacity [394]. Similarly, electrophysiological indicators of cell damage like depolarization of resting membrane potential or loss of input resistance are blunted.

Changes in expression of various CaPs have been reported in a number of neurodegenerative diseases [395]. In patients with Down's syndrome, the CaP S100β is increased whereas immunoreactive staining of calbindin D28K and parvalbumin is reduced in cortical neurons [396]. In Parkinson's disease, mRNA and protein levels of calbindin D28K are decreased in various brain regions [397]. In Alzheimer's disease (AD) a decrease in calmodulin [398] and an increase in S100 protein have been described [399].

According to studies on neuronal cells or on cells of neuroectodermal origin, like skin fibroblasts, in AD the intracellular Ca^{2+} handling is impaired. Early observations described a decreased basal $[Ca^{2+}]_i$ and a decreased response to hormonal stimulus in fibroblasts; however, the total Ca^{2+} content of the cells was increased [400]. In addition, an altered activity of the plasma membrane Ca^{2+}-ATPase has been reported. In contrast, later observations suggested that Ca^{2+} mobilization is enhanced in AD due to the presence of the β-amyloid protein which represents the main component of the senile plaques. In several cellular models, incubation with synthetic β-amyloid (fragments amino acid 1–38 or 25–35) increased resting Ca^{2+} levels as well as Ca^{2+} response to depolarization or to exposure of Ca^{2+} ionophores [401–403]. Furthermore, these fragments enhanced the neurotoxicity of the excitatory amino acids as well as the calcium-linked degenerative effect of excitoxicity [404].

β-amyloid itself might act as an additional Ca^{2+} channel, mediating influx into the cells [405]. Increased $[Ca^{2+}]_i$, when present over an extended period of time, should evoke cellular adaptations. In fact, an enhanced activity of the Na^+/Ca^{2+} exchanger has been demonstrated in cerebral plasma membrane vesicles of AD patients [210].

The involvement of Ca^{2+} in the pathophysiological cascade of AD has been suggested also by measurements of Ca^{2+}-activated proteinases (calpain). Their activity has been shown to be increased in the brain of deceased AD patients [215]. Likewise, their degrading capacity could, at least in part, link elevation of cell Ca^{2+} to cellular degeneration.

4. The transition from physiological to pathophysiological states in calcium handling: some general principles

In Table 3, some essential features of disease processes with regard to basal intracellular Ca^{2+},

Table 3
Multiple scenarios of altered cellular Ca^{2+} homeostasis underlying different diseases

$[Ca^{2+}]_i$ basal	$[Ca^{2+}]_i$ response to stimulation	Example	Type of disease	Reference
Steadily increasing	$\Rightarrow / \Downarrow$ blunted by high intracellular Ca^{2+}	infarction (p. 32) toxin-induced cell death (p. 27) acute pancreatitis (p. 28)	acute	[270,276,385]
Increased, stable	$\Uparrow$	hypertension (p. 36)	chronic	[336,338]
	$\Downarrow$	idiopathic heart failure (p. 33)	chronic	[320,321]
Normal, stable	$\Uparrow$	Alzheimer's disease (p. 41)	chronic	[401]
	$\Downarrow$	chronic inflammatory diseases (Crohn's disease, rheumatoid arthritis) (p. 30)	chronic	[295,296]

response of intracellular Ca^{2+} to physiological stimuli and the intracellular Ca^{2+} buffer capacity, are compiled. When comparing the different diseases, three different conditions can be distinguished: first, resting $[Ca^{2+}]_i$ is increasing continuously, finally resulting in cell death initiating the acute disease. Ca^{2+} controlling systems are no longer able to maintain $[Ca^{2+}]_i$ in a steady-state. This behaviour is observed in acute diseases like infarction or after exposure to chemical or biological toxins. Second, resting $[Ca^{2+}]_i$ is increased to a higher baseline; however, the increased level is more or less stable over a certain period of time, i.e., $[Ca^{2+}]_i$ is still controlled although at a higher setpoint. This type of Ca^{2+} imbalance is often found in hypertension and in chronic diseases. And third, basal $[Ca^{2+}]_i$ levels are normal.

With regard to the Ca^{2+} response to physiological stimuli, a pathological status may result in increased or decreased Ca^{2+} responses. If the response is increased, cellular hyperactivity can ensue as in hypertension or chronic inflammatory diseases. Such hyperactivity always carries the risk that when the intracellular Ca^{2+} buffer capacity or the mechanisms to remove Ca^{2+} from the cytosol are impaired, intracellular Ca^{2+} might rise to levels where cellular degradation is initiated.

Which mechanisms support an enhanced Ca^{2+} responsiveness? Under physiological conditions, the Ca^{2+} signal is 'under control' (i.e., reaches 0.5 to 1.5 $\mu M/l$) and does not exceed a certain threshold. Ca^{2+} buffers play an important role in this protection of the cell since they absorb huge amounts of Ca^{2+}. The dissociation constants cover nearly the whole range of the physiological Ca^{2+} signals. Assuming that functional buffer capacity (i.e., intracellular buffers and Ca^{2+} removing mechanism) is normal, as symbolized by the box in Fig. 5, activation of harmful processes by free Ca^{2+} is prevented.

On the left hand side of Fig. 5 a situation is depicted where the cellular Ca^{2+} response to stimuli is enhanced. This may be caused by an increased Ca^{2+} influx or enhanced release of Ca^{2+} from intracellular stores. If the Ca^{2+} entering the cytosol exceeds the Ca^{2+} buffer capacity of the cell, the free Ca^{2+} concentration can rise above the physiological threshold, thereby inducing cellular hyperactivity or reactions potentially detrimental to the cell.

Cellular responsiveness can also be enhanced when intracellular Ca^{2+} buffer capacity is reduced as illustrated on the lower right hand side of Fig. 5. In this instance, a physiological Ca^{2+} signal might be sufficient to generate Ca^{2+} levels that exceed the threshold. This may be called a relative Ca^{2+} overload. If in such a situation the Ca^{2+} influx caused by stimulation is increased, nondetrimental levels of $[Ca^{2+}]_i$

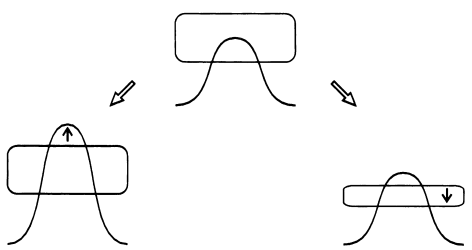

Fig. 5. Schematic diagram demonstrating the relation of intracellular free Ca^{2+} levels and different intracellular Ca^{2+} buffering capacities (CBC). Increased Ca^{2+} signals higher than CBC can induce degrading processes (left). Normal Ca^{2+} signals may give the same result when intracellular CBC is decreased (right).

can be exceeded relatively easily. During ischemia and inflammation, a decrease of intracellular Ca^{2+} buffer capacity has been observed. In hypertension, functional buffer capacity of the cell appears also to be decreased by the reduction in calmodulin and a reduced rate of Ca^{2+} extrusion from the cells.

Thus far, however, only a few studies have confirmed a protective role of Ca^{2+} buffers in disease processes. One study demonstrated that cells containing higher amounts of Ca^{2+} binding proteins are more resistant to harmful pathological stimuli; in the other case, an increase of Ca^{2+} buffer capacity by application of a Ca^{2+} chelator resulted in a lower incidence of postinfarction arrythmias [319].

It should be noted at this point that an important factor for the effectiveness of potentially harmful Ca^{2+} signals is time. Time and extent of Ca^{2+} signal seem to be inversely related in initiating cell damage. That means, large changes of $[Ca^{2+}]_i$ are acutely effective in inducing cell death, e.g., after toxin application. However, the same effect may be obtained by chronic exposure to a small but permanent Ca^{2+} overload.

Under several pathological conditions, a reduced response of intracellular Ca^{2+} to stimuli is observed. This leads to a functional defect of the cells. Thereby, basal $[Ca^{2+}]_i$ is only sometimes affected, whereas Ca^{2+} mobilization upon stimulation is clearly suppressed. The origin of this phenomenon is unclear. It may be that a state of decreased response follows a phase of increased Ca^{2+} responsiveness. Such decreased Ca^{2+} responses also might be due to disturbed signalling pathways. In this context, a decreased activation of G-proteins, decreased levels of IP_3 or lower responsiveness of intracellular Ca^{2+} channels might be potential pathological mechanisms.

These alterations can also be caused by a reduced refilling of intracellular stores, a more efficient Ca^{2+} extrusion, or both. Expression of Ca^{2+} sequestering systems may be downregulated or their activity may be changed by phosphorylation/dephosphorylation processes. Furthermore, an upregulation of a Ca^{2+} extrusion system, such as the Na^+/Ca^{2+} exchanger, has been reported.

Although cells with decreased Ca^{2+} availability demonstrate an impaired functionality, this does not necessarily mean that cell viability in general is impaired. The increased or reduced cellular functionality may precede the loss of cellular integrity, for a more or less extended time period, which is part of a chronic disease.

It should be noted that all the changes described above for the cellular level also have implications for the function of whole organs and even the life of a patient. The extent of impairment depends thereby on the availability and effectivity of repair mechanisms as well as the duration of the alteration. In particular, the disturbance in cellular Ca^{2+} might not lead to the death of the afflicted cell in chronic diseases, but the failure of body function might ultimately be caused by secondary effects on other vital organ systems.

5. Epilogue

Thus, in acute and chronic diseases, severe disturbances in Ca^{2+} handling by the affected cells are observed. This contribution attempted to deduce some basic principles to explain some of these alterations. In order to do so, the complex in vivo situation had to be simplified considerably. Nevertheless, the authors hope that their approach will stimulate studies on this important subject that lead to a deeper insight into the cellular and molecular basis of disease processes.

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References

[1] H. Davy, Philos. Trans. R. Soc. London 98 (1808) 333–370.
[2] S. Ringer, Practitioner 31 (1883) 81–93.
[3] S. Ringer, J. Physiol. (London) 4 (1883) 29–43.
[4] S. Ringer, J. Physiol. (London) 11 (1890) 79–84.
[5] H. Lawaczek, Dtsch. Arch. Klin. Med. 160 (1928) 302–309.
[6] S. Hermann, Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 167 (1932) 82–84.
[7] E. Weise, Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 176 (1934) 367–377.

[8] Th. Wense, Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol. 176 (1934) 49–58.
[9] H. Pollack, J. Gen. Physiol. 11 (1928) 539–545.
[10] E.B. Ridgway, C.C. Ashley, Biochem. Biophys. Res. Commun. 29 (1967) 229–234.
[11] A. Scarpa, F.J. Brinley, T. Tiffert, G.R. Dubyak, Ann. New York Acad. Sci. 307 (1978) 86–112.
[12] D. Ammann, P.C. Meier, W. Simon, in: C.C. Ashley, A.K. Campbell (Eds.), Detection and Measurement of Free Ca^{2+} in Cells, Elsevier, Amsterdam, 1979, pp. 117–129.
[13] G. Grynkiewicz, M. Poenie, R.Y. Tsien, J. Biol. Chem. 260 (1985) 3440–3450.
[14] R.Y. Tsien, Biochemistry 19 (1980) 2396–2404.
[15] D.L. Thompson, J.B. Collip, Physiol. Rev. 12 (1932) 309–383.
[16] M. Kohlhardt, B. Bauer, H. Krause, A. Fleckenstein, Pflug. Arch. Eur. J. Physiol. 335 (1972) 309–322.
[17] F. Murad, in: A. Goodman, Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 1992, pp. 764–783
[18] J.A. Fleckenstein, H. Kammermeier, H. Doring, H.J. Freund, Z. Kreislaufforsch. 56 (1967) 839–858.
[19] R.A. Hickie, J. Wie, L.M. Blyth, D.Y.W. Wong, Can. J. Biochem. Cell Biol. 61 (1983) 934–941.
[20] R.R. Ratan, M.L. Shelanski, Trends Biochem. Sci. 11 (1986) 456–459.
[21] M. Poenie, J. Alderton, R.Y. Tsien, R.A. Steinhardt, Nature 315 (1985) 147–149.
[22] A.K. Campbell (Ed.), Intracellular Calcium, Wiley, Chichester, 1983, pp. 135–205.
[23] C.S. Farah, F.C. Reinach, FASEB J. 9 (1995) 755–767.
[24] C. Christensen, Nature 330 (1987) 66–68.
[25] F.Ch. Mooren, R.K.H. Kinne, Pflug. Arch. Eur. J. Physiol. 427 (1994) 463–472.
[26] P. Thomas, A. Surprenant, W. Almers, Neuron 5 (1990) 723–733.
[27] E. Carafoli, Annu. Rev. Biochem. 56 (1987) 395–433.
[28] S. Muallem, Annu. Rev. Physiol. 51 (1989) 83–105.
[29] L.M. Schwartz, J. Neurobiol. 23 (1992) 1312–1326.
[30] M.J. Arends, R.G. Morris, A.H. Wyllie, Am. J. Pathol. 136 (1990) 593–608.
[31] D.J. McConkey, S. Orrenius, J. Leukocyte Biol. 59 (1996) 775–783.
[32] P.A. Friedman, F.A. Gesek, Physiol. Rev. 75 (1995) 429–471.
[33] R.H. Wassermann, C.S. Fullmer, J. Nutr. 125 (1995) 1971S–1979S.
[34] T. Pozzan, R. Rizzuto, P. Volpe, J. Meldolesi, Physiol. Rev. 74 (1994) 595–636.
[35] J.K.H. Rees, H.A. Coles, Br. Med. J. 2 (1969) 670–672.
[36] E. Chanat, W.B. Huttner, J. Cell. Biol. 115 (1991) 1505–1519.
[37] C. Fasolato, M. Zottini, E. Clementi, D. Zachetti, J. Meldolesi, T. Pozzan, J. Biol. Chem. 266 (1991) 20159–20167.
[38] H. Streb, E. Bayerdorffer, W. Haase, R.F. Irvine, I. Schulz, J. Membr. Biol. 81 (1984) 241–253.
[39] D. Milani, A. Malgaroli, D. Guidolin, C. Fasolato, S.D. Skaper, J. Meldolesi, T. Pozzan, Cell Calcium 11 (1990) 191–199.
[40] K.H. Krause, FEBS Lett. 285 (1991) 225–229.
[41] P. Volpe, K.-H. Krause, S. Hashimoto, F. Zorvato, T. Pozzan, J. Meldolesi, D.P. Lew, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 1091–1095.
[42] R.D. Burgoyne, T.R. Cheek, A. Morgan, A.J. O'Sullivan, R.B. Moreton, M.J. Berridge, A.M. Mata, J. Colyer, A.G. Lee, J.M. East, Nature 342 (1989) 72–74.
[43] V.A. Golovina, M.P. Blaustein, Science 275 (1997) 1643–1648.
[44] H. Kasai, Y.X. Li, Y. Miyashita, Cell 74 (1993) 669–677.
[45] O.H. Petersen, C.C.H. Petersen, H. Kasai, Annu. Rev. Physiol. 56 (1994) 297–319.
[46] G. Hajnoczky, C. Lin, A.P. Thomas, J. Biol. Chem. 269 (1994) 10280–10287.
[47] L. Wu, S. Katz, G.R. Brown, Cell Calcium 15 (1994) 228–240.
[48] B. Brüne, V. Ullrich, J. Biol. Chem. 266 (1991) 19232–19237.
[49] A.H. Guse, E. Roth, F. Emmrich, Biochem. J. 291 (1993) 447–451.
[50] H.C. Lee, R. Aarhus, J. Biol. Chem. 270 (1995) 2152–2157.
[51] J. Meldolesi, L. Madeddu, T. Pozzan, Biochim. Biophys. Acta 1055 (1990) 130–140.
[52] S.E. Cala, C. Ulbright, J.S. Kelley, L.R. Jones, J. Biol. Chem. 268 (1992) 2969–2975.
[53] M. Michalak, L. Fliegel, K. Wlasichuk, J. Biol. Chem. 265 (1990) 5869–5874.
[54] L. Fliegel, K. Burns, M. Opas, M. Michalak, Biochim. Biophys. Acta 982 (1989) 1–8.
[55] R.E. Milner, S. Baksh, C. Shemanko, M.R. Carpenter, L. Smilie, J.E. Vance, M. Opas, M. Michalak, J. Biol. Chem. 266 (1991) 7155–7165.
[56] P.D. Nash, M. Opas, M. Michalak, Mol. Cell. Biochem. 135 (1994) 71–78.
[57] M. Michalak, S. Baksh, M. Opas, Exp. Cell Res. 197 (1991) 91–99.
[58] S. Baksh, M. Michalak, J. Biol. Chem. 266 (1991) 21458–21465.
[59] O. Blondel, M.M. Moody, A.M. Depaoli, A.H. Sharp, C.A. Ross, H. Swift, G.I. Bell, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 7777–7781.
[60] O.V. Gerasimenko, J.V. Gerasimenko, P.V. Belan, O.H. Petersen, Cell 84 (1996) 473–480.
[61] H. Kasai, O.H. Petersen, Trends Neurosci. 17 (1994) 95–101.
[62] H. Mogami, T. Nakano, A.V. Tepikin, O.H. Petersen, Cell 88 (1997) 49–55.
[63] K. Mikoshiba, Trends Physiol. Sci. 14 (1993) 86–89.
[64] R.H. Ashley, Essays Biochem. 30 (1995) 97–117.

[65] B.E. Ehrlich, E. Kaftan, S.G. Bezprozvanny, I. Bezprozvanny, Trends Physiol. Sci. 15 (1994) 145–149.
[66] O. Blondel, J. Takeda, H. Janssen, S. Seino, G.I. Bell, J. Biol. Chem. 268 (1993) 11356–11363.
[67] D. Restropo, Science 249 (1991) 1166–1168.
[68] M. Kuno, P. Gardner, Nature 326 (1987) 301–304.
[69] S. Supattapone, P.F. Worley, J.N. Baraban, S.H. Snyder, J. Biol. Chem. 263 (1988) 1530–1534.
[70] P. Champeil, L. Combettes, B. Berthon, E. Doucet, S. Orlowski, M. Claret, J. Biol. Chem. 264 (1989) 17665–17673.
[71] T. Meyer, T. Wensel, L. Stryer, Biochemistry 29 (1990) 32–37.
[72] I. Bezprozvanny, S.G. Bezprozvanny, B.E. Ehrlich, Mol. Biol. Cell 5 (1994) 97–103.
[73] C.D. Ferris, R.L. Huganir, D.S. Bredt, A.M. Cameron, S.H. Snyder, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 2232–2235.
[74] X. Xu, W. Zeng, S. Muallem, J. Biol. Chem. 271 (1996) 11737–11744.
[75] I. Bezprozvanny, J. Watras, B.E. Ehrlich, Nature 351 (1991) 751–754.
[76] L. Missiaen, H. De Smedt, G. Droogmans, R. Casteels, Nature 357 (1992) 599–602.
[77] V. Sorrentino, P. Volpe, Trends Physiol. Sci. 14 (1993) 98–103.
[78] G. Meissner, Annu. Rev. Physiol. 56 (1994) 485–508.
[79] R. Coronado, J. Morrissette, M. Sukhareva, D.M. Vaughan, Am. J. Physiol. 266 (1994) C1485–C1504.
[80] E. Rios, G. Brum, Nature 325 (1987) 717–720.
[81] H. Takeshima, S. Nishimura, T. Matsumoto, H. Ishida, K. Kangawa, N. Minamino, H. Matsuo, M. Ueda, M. Hanaoka, T. Hirose, Nature 339 (1989) 439–445.
[82] G. Giannini, A. Conti, S. Mammarella, M. Scobogna, V. Sorrentino, J. Cell Biol. 128 (1995) 893–904.
[83] J.S. Smith, T. Imagawa, J. Ma, M. Fill, K.P. Campbell, R. Coronado, J. Gen. Physiol. 92 (1988) 1–26.
[84] R. Sitsapesan, A.J. Williams, J. Membr. Biol. 137 (1994) 215–226.
[85] D.R. Witcher, R.J. Kovacs, H. Schulman, D.C. Cefali, L.R. Jones, J. Biol. Chem. 266 (1991) 11144–11152.
[86] J.S. Smith, E. Rousseau, G. Meissner, Circ. Res. 64 (1989) 352–359.
[87] J.S. Smith, R. Coronado, G. Meissner, J. Gen. Physiol. 88 (1986) 573–588.
[88] H.H. Valdivia, M.S. Kirby, W.J. Lederer, R. Coronado, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 12185–12189.
[89] A. Galione, H.C. Lee, W.B. Busa, Science 253 (1991) 1143–1146.
[90] R. Sitsapesan, S.J. McGarry, A.J. Williams, Trends Physiol. Sci. 16 (1995) 386–391.
[91] H.C. Lee, R. Aarhus, R.M. Graef, J. Biol. Chem. 270 (1995) 9060–9066.
[92] A. Galione, A. White, Trends Cell. Biol. 4 (1994) 431–436.
[93] M. Diaz-Munoz, S.L. Hamilton, M.A. Kaetzel, P. Hazarika, J.R. Dedman, J. Biol. Chem. 265 (1990) 15894–15899.
[94] E. Kaftan, A.R. Marks, B.E. Ehrlich, Circ. Res. 78 (1996) 990–997.
[95] M. Mayrleitner, A.P. Timerman, G. Wiederrecht, S. Fleischer, Cell Calcium 15 (1994) 99–108.
[96] R. Penner, C. Fasolato, M. Hoth, Curr. Opin. Neurobiol. 3 (1993) 368–374.
[97] J. Meldolesi, T. Pozzan, Exp. Cell Res. 171 (1987) 271–283.
[98] M. Estacion, L.J. Mordan, Cell Calcium 14 (1993) 439–454.
[99] E. Carbone, H.D. Lux, Nature 310 (1984) 501–502.
[100] N. Hagiwara, H. Irisawa, M. Kameyama, J. Physiol. (London) 395 (1988) 233–253.
[101] T.F. McDonald, S. Pelzer, W. Trautwein, D.J. Pelzer, Physiol. Rev. 74 (1994) 365–507.
[102] M.C. Nowycky, A.P. Fox, R.W. Tsien, Nature 316 (1985) 440–443.
[103] P. Hess, J.B. Lansman, R.W. Tsien, Nature 311 (1984) 538–544.
[104] L.D. Hirning, A.P. Fox, E.W. McCleskey, B.M. Olivera, S.A. Thayer, R.J. Miller, R.W. Tsien, Science 239 (1988) 57–61.
[105] W. Trautwein, A. Cavalie, T.J.A. Allen, Y.M. Shuba, S. Pelzer, D. Pelzer, in: Y. Nishizuka (Ed.), The Biology and Medicine of Signal Transduction, Raven Press, New York, 1990, pp.
[106] G.-N. Tseng, P.A. Boyden, Am. J. Physiol. 261 (1991) H364–H379.
[107] R.L. Rosenberg, P. Hess, J.P. Reeves, H. Smilowitz, R.Y. Tsien, Science 231 (1986) 1564–1566.
[108] R.L. Rosenberg, P. Hess, R.Y. Tsien, J. Gen. Physiol. 92 (1988) 27–54.
[109] D. Armstrong, R. Eckert, Proc. Natl. Acad. Sci. U.S.A. 84 (1987) 2518–2522.
[110] D. Pelzer, A.O. Grant, A. Cavalie, S. Pelzer, M. Sieber, F. Hofmann, W. Trautwein, Ann. New York Acad. Sci. 560 (1989) 138–154.
[111] W.A. Catterall, J. Striessnig, Trends Pharmacol. Sci. 13 (1992) 256–262.
[112] R.J. Miller, J. Biol. Chem. 267 (1992) 1403–1406.
[113] S. Kongsamut, D. Lipscombe, R.W. Tsien, Ann. New York Acad. Sci. 500 (1989) 312–333.
[114] D. Lipscombe, D.V. Madison, M. Poenie, H. Reuter, R.Y. Tsien, R.W. Tsien, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 2398–2402.
[115] T.J. Rink, FEBS Lett. 268 (1990) 381–385.
[116] R.W. Tsien, R.Y. Tsien, Annu. Rev. Cell Biol. 6 (1990) 715–760.
[117] C.D. Benham, R.W. Tsien, Nature 328 (1987) 275–278.

[118] D.D. Friel, B.P. Bean, J. Gen. Physiol. 91 (1988) 1–27.
[119] R.I. Hume, M.G. Honig, J. Neurosci. 6 (1986) 681–690.
[120] B.P. Bean, J. Neurosci. 10 (1990) 1–10.
[121] S.R. Alonso-Torre, A. Trautmann, J. Biol. Chem. 268 (1993) 18640–18647.
[122] P. Vincent, J. Physiol. (London) 449 (1992) 313–331.
[123] R. Penner, G. Matthews, E. Neher, Nature 334 (1988) 499–504.
[124] M. Hoth, R. Penner, J. Physiol. (London) 465 (1993) 359–386.
[125] A.P. Morris, D.V. Gallacher, R.F. Irvine, O.H. Petersen, Nature 330 (1987) 653–655.
[126] M.J. Berridge, Biochem. J. 312 (1995) 1–11.
[127] P.M. Snyder, K.-H. Krause, M.J. Welsh, J. Biol. Chem. 263 (1988) 11048–11051.
[128] A. Lückhoff, D.E. Clapham, Nature 355 (1992) 356–358.
[129] N. Demaurex, D.P. Lew, K.H. Krause, J. Biol. Chem. 267 (1992) 2318–2324.
[130] L. Vaca, D.L. Kunze, Am. J. Physiol. 267 (1994) C920–C925.
[131] B. Premack, T.V. McDonald, P. Gardner, J. Immunol. 152 (1994) 5226–5240.
[132] J.W. Putney Jr., Cell Calcium 7 (1986) 1–12.
[133] J.W. Putney Jr., Curr. Top. Cell. Regul. 31 (1990) 111–127.
[134] G.St.J. Bird, M.F. Rossier, A.R. Hughes, S.B. Shears, D.L Armstrong, J.W. Putney Jr., Nature 352 (1991) 162–165.
[135] H. Takemura, A.R. Hughes, O. Thastrup, J.W. Putney Jr., J. Biol. Chem. 264 (1989) 1226–12271.
[136] M. Hoth, R. Penner, Nature 355 (1992) 353–356.
[137] T.V. McDonald, B.A. Premack, P. Gardner, J. Biol. Chem. 268 (1993) 3889–3896.
[138] C. Randriamampita, R.Y. Tsien, Nature 364 (1993) 809–814.
[139] A.B. Parekh, H. Terlau, W. Stühmer, Nature 364 (1993) 814–818.
[140] C. Fasolato, M. Hoth, R. Penner, J. Biol. Chem. 268 (1993) 20737–20740.
[141] G.St.J. Bird, J.W. Putney Jr., J. Biol. Chem. 268 (1993) 21468–21488.
[142] S.J. Pandol, A. Gukovskaya, T.D. Bahnson, V.E. Dionne, Ann. New York Acad. Sci. 713 (1994) 41–48.
[143] P. Sargeant, R.W. Farndale, S.O. Sage, FEBS Lett. 315 (1993) 242–246.
[144] D.I. Yule, E.T. Kim, J.A. Williams, Biochem. Biophys. Res. Commun. 202 (1994) 1697–1704.
[145] S. Jenner, R.W. Farndale, S.O. Sage, Biochem. J. (1994) 337–339.
[146] X. Xu, R.A. Star, C. Tortorici, S. Muallem, J. Biol. Chem. 269 (1994) 12645–12653.
[147] R.C. Hardie, B. Minke, Trends Neurosci. 16 (1993) 371–376.
[148] D.L. Bennett, C.C.H. Petersen, T.R. Cheek, Curr. Biol. 5 (1995) 1225–1228.
[149] R.C. Hardie, B. Minke, Neuron 8 (1992) 643–651.
[150] F. Sachs, Membr. Biochem. 6 (1986) 173–195.
[151] X.-C. Yang, F. Sachs, Science 243 (1989) 1068–1071.
[152] C. Fasolato, M. Hoth, R. Penner, Pflug. Arch. Eur. J. Physiol. 423 (1993) 225–231.
[153] E. Carafoli, J. Biol. Chem. 267 (1992) 2115–2118.
[154] A.K. Grover, I. Khan, Cell Calcium 13 (1992) 9–17.
[155] C.J. Brandl, N.M. Green, B. Korczak, D.H. MacLennan, Cell 44 (1986) 597–607.
[156] S.E. Burk, J. Lytton, D.H. MacLennan, G.E. Shull, J. Biol. Chem. 264 (1989) 18561–18568.
[157] C. Brandl, S. de Leon, D. Martin, D.H. MacLennan, J. Biol. Chem. 262 (1987) 3768–3774.
[158] M.M. Thelen, A. Muller, M.J. Zuidwijk, G.C. van der Linden, W.S. Simonides, C. van Hardefeld, Biochem. J. 303 (1994) 467–474.
[159] A.M. Campbell, P.D. Kessler, D.M. Fambrough, J. Biol. Chem. 267 (1992) 9321–9325.
[160] D.M. Clarke, T.W. Loo, G. Inesi, D.H. MacLennan, Nature 339 (1989) 476–478.
[161] J. Lytton, M. Westlin, S.E. Burk, G.E. Shull, D.H. MacLennan, J. Biol. Chem. 267 (1992) 14483–14489.
[162] H. Verboomen, F. Wuytack, L. van den Bosch, L. Mertens, R. Casteels, Biochem. J. 303 (1994) 979–984.
[163] O. Thastrup, H. Linneberg, P.J. Bjerrum, J.B. Knudsen, S.B. Christensen, Biochim. Biophys. Acta 927 (1987) 65–73.
[164] O. Thastrup, P.J. Cullen, B. Drobak, M.R. Hanley, A.P. Dawson, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 2466–2470.
[165] S. Hechtenberg, D. Beyersmann, Enzyme 45 (1991) 109–115.
[166] P.M. Vrbost, M.H.M.N. Senden, C.H. van Os, Biochim. Biophys. Acta 902 (1987) 247–252.
[167] E. Strehler, M. Strehler-Page, G. Vogel, E. Carafoli, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 6908–6912.
[168] J. Greeb, G. Shull, J. Biol. Chem. 264 (1989) 18569–18576.
[169] B. Sarkadi, A. Enyedi, G. Gardos, Cell Calcium 1 (1980) 287–298.
[170] P. Caroni, E. Carafoli, J. Biol. Chem. 256 (1981) 9371–9373.
[171] H.W. Jarrett, J.T. Penniston, Biochem. Biophys. Res. Commun. 77 (1977) 1210–1216.
[172] A. Enyedi, T. Vorherr, P. James, D.J. McCormick, A.G. Filoteo, E. Carafoli, J.T. Penniston, J. Biol. Chem. 264 (1989) 12313–12316.
[173] V. Niggli, E.S. Adunyah, J.T. Penniston, E. Carafoli, J. Biol. Chem. 256 (1981) 395–401.
[174] B. Zhang, H. Zhao, P. Loessberg, S. Muallem, J. Biol. Chem. 267 (1992) 15419–15425.
[175] H. Ariyoshi, E. Shiba, J. Kambayashi, M. Sakon, T. Kawasaki, K. Yoshida, T. Mori, Cell Calcium 14 (1993) 455–463.

[176] P. James, T. Vorherr, J. Krebs, A. Morelli, G. Castello, D.J. McCormick, J.T. Penniston, A. De Flora, E. Carafoli, J. Biol. Chem. 264 (1989) 8289–8296.
[177] P.F. Baker, M.P. Blaustein, A.L. Hodgkin, R.A. Steinhardt, J. Physiol. (London) 200 (1969) 431–458.
[178] H. Reuter, N. Seutz, J. Physiol. (London) 195 (1968) 451–470.
[179] H. Reuter, M.P. Blaustein, G. Hausler, Philos. Trans. R. Soc. London V265 (1973) 87–94.
[180] P. Kofuji, R.W. Hadley, R.S. Kieval, W.J. Lederer, D.H. Schulze, Am. J. Physiol. 263 (1992) C1241–C1249.
[181] E. Bayerdörffer, W. Haase, I. Schulz, J. Membr. Biol. 87 (1985) 107–119.
[182] P. Kofuji, W.J. Lederer, D.H. Schulze, Am. J. Physiol. 265 (1993) F598–F603.
[183] K.D. Philipson, D.A. Nicoll, S. Matsuoha, L.V. Hryshko, D.O. Levitsky, J.N. Weiss, Ann. New York Acad. Aci. 779 (1996) 20–28.
[184] K.D. Philipson, S.L. Longoni, R. Ward, Biochim. Biophys. Acta 945 (1988) 298–306.
[185] D.A. Nicoll, K.D. Philipson, Ann. New York Acad. Sci. 639 (1991) 181–188.
[186] J.P. Reeves, G. chernaya, M. Condrescu, Ann. New York Acad. Sci. 779 (1996) 73–85.
[187] P.A. McNaughton, Ann. New York Acad. Sci. 639 (1991) 2–9.
[188] Z. Li, E.P. Burke, J.s. Frank, V. Bennett, K.D. Philipson, J. Biol. Chem. 268 (1993) 11489–11491.
[189] R. DiPolo, L. Beauge, Ann. New York Acad. Sci. 639 (1991) 100–111.
[190] J. Kimura, Ann. New York Acad. Sci. 779 (1996) 515–520.
[191] T.L. Trosper, K. Philipson, Biochim. Biophys. Acta 731 (1983) 63–68.
[192] P.F. Baker, P.A. McNaughton, J. Physiol. (London) 269 (1977) 78p–79p.
[193] A.E. Doering, W.J. Lederer, J. Physiol. (London) 466 (1993) 481–499.
[194] A.E. Doering, W.J. Lederer, J. Physiol. (London) 480 (1994) 9–20.
[195] A.E. Doering, D.A. Eisner, W.J. Lederer, Ann. New York Acad. Sci. 779 (1996) 182–198.
[196] J.P. Reeves, C.C. Hale, J. Biol. Chem. 259 (1984) 7733–7739.
[197] L. Lagnado, P.A. McNaughton, J. Membr. Biol. 113 (1990) 177–191.
[198] J.R. Berlin, M.B. Cannell, W.J. Lederer, Am. J. Physiol. 253 (1987) H1540–H1547.
[199] A.J. Levi, P. Brooksby, J.C. Hancox, Cardiovasc. Res. 27 (1993) 1743–1757.
[200] A.J. Levi, K.W. Spritzer, J.H.B. Bridge, O. Kohnoto, Am. J. Physiol. 266 (1994) H1422–H1433.
[201] W.J. Lederer, S. He, S. Luo, W. DuBell, P. Kofuji, R. Kieval, C.F. Neubauer, A. Ruknudin, H. Cheng, M.B. Cannel, T.B. Rogers, D.H. Schulze, Ann. New York Acad. Sci. 779 (1996) 7–16.
[202] P. Gmaj, H. Murer, R. Kinne, Biochem. J. 178 (1979) 549–557.
[203] M.P. Blaustein, W.F. Goldman, G. Fontana, B.K. Krueger, E.M. Santiago, T.D. Steele, D.N. Weiss, P.I. Yarowsky, Ann. New York Acad. Sci. 639 (1991) 254–274.
[204] E. Neher, G.J. Augustine, J. Physiol. (London) 450 (1992) 273–301.
[205] A.K. Campbell (Ed.), Intracellular Calcium, Wiley, Chichester, 1983, pp. 120–123.
[206] E.P. Abraham, in: H.W. Lord (Ed.), General Pathology, Lloyd–Luke, London, 1970, pp. 431–450.
[207] V. von Tscharner, D.A. Deranleau, M. Baggiolini, J. Biol. Chem. 261 (1986) 10163–10168.
[208] A.K. Campbell (Ed.), Intracellular Calcium, Wiley, Chichester, 1983, pp. 104–114.
[209] C.S. Farah, F. Reinach, FASEB J. 9 (1995) 755–767.
[210] R.A. Calvin, J.W. Bennett, S.L. Colvin, Ann. New York Acad. Sci. 639 (1991) 325–327.
[211] B. Bruns, J.M. McDonald, L. Jarett, J. Biol. Chem. 252 (1977) 927–932.
[212] V. Gerke, K. Weber, EMBO J. 3 (1984) 227–233.
[213] T.C. Sudhof, J.H. Walker, J. Obrocki, EMBO J. 1 (1982) 1167–1170.
[214] T.C. Sudhof, M. Ebbecke, J.H. Walker, U. Fritsche, C. Boustead, Biochemistry 23 (1984) 1103–1109.
[215] K. Saito, J.S. Elce, J.E. Hamos, R.A. Nixon, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 2628–2632.
[216] M.A. Markus, T. Nakayama, P. Matsudaira, G. Wagner, Protein Sci. 3 (1994) 70–81.
[217] R.H. Kretsinger, Annu. Rev. Biochem. 45 (1976) 239–268.
[218] Y. Saimi, C. Kung, FEBS Lett. 350 (1994) 155–158.
[219] A.C. Nairn, in: Y. Nishizuka (Ed.), The Biology and Medicine of Signal Transduction, Raven Press, New York, 1990, pp. 202–205.
[220] H.J. Vogel, Biochem. Cell Biol. 72 (1994) 357–376.
[221] F. Bronner, Miner. Electrolyte Metabol. 16 (1990) 94–100.
[222] B.B. Olwin, D.R. Storm, Biochemistry 24 (1985) 8081–8086.
[223] M.J. Geisow, J.H. Walker, Trends Biochem. Sci. 11 (1986) 420–423.
[224] C.B. Klee, Biochemistry 27 (1988) 6645–6653.
[225] J.R. Glenney Jr., Biochem. Soc. Trans. 15 (1987) 798–800.
[226] S.T. Sawyer, S. Cohen, J. Biol. Chem. 260 (1985) 8233–8236.
[227] D.S. Drust, C.E. Creutz, Nature 331 (1988) 88–91.
[228] M.J. Geisow, J.H. Walker, C. Boustead, W. Taylor, Biochem. Soc. Trans. 15 (1987) 800–802.
[229] E.F. Etter, M.A. Kuhn, F.S. Fay, J. Biol. Chem. 269 (1994) 10141–10149.
[230] E. Melloni, S. Pontremoli, Trends Neurosci. 12 (1989) 438–444.

[231] P. Johnson, Int. J. Biochem. 22 (1990) 811–822.
[232] F.J. Brinley Jr., Annu. Rev. Biophys. Bioeng. 7 (1978) 363–392.
[233] F.J. Brinley Jr., T. Tiffert, A. Scarpa, J. Gen. Physiol. 72 (1078) 101–127.
[234] R. Rizzuto, A.W.M. Simpson, M. Brini, T. Pozzan, Nature 358 (1992) 325–327.
[235] T.E. Gunter, D.R. Pfeiffer, Am. J. Physiol. 258 (1990) C755–C786.
[236] T.E. Gunter, K.K. Gunter, S. Sheu, C.E. Gavin, Am. J. Physiol. 267 (1994) C313–C339.
[237] Y. Tsunoda, E.L. Stuenkel, J.A. Williams, Am. J. Physiol. 259 (1990) G792–G801.
[238] M.J. Berridge, R.F. Irvine, Nature 312 (1984) 315–321.
[239] A. Parekh, H. Terlau, Pflug. Arch. Eur. J. Physiol. 432 (1996) 14–25.
[240] C. Franzini-Armstrong, A.O. Jorgensen, Annu. Rev. Physiol. 56 (1994) 509–534.
[241] A.B. Borle, Rev. Physiol. Biochem. Pharmacol. 90 (1981) 13–153.
[242] T. Meyer, Cell 64 (1991) 675–678.
[243] T. Takamatsu, W.G. Wier, FASEB J. 4 (1990) 1519–1525.
[244] J. Lechleiter, S. Girard, E. Perala, D. Clapham, Science 252 (1991) 123–126.
[245] L.F. Jaffe, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 9883–9887.
[246] P.H. Backx, P.P. De Tombe, J.H.K. van Deen, B.J.M. Mulder, H.E.D.J. Ter Keus, J. Gen. Physiol. 93 (1989) 963–977.
[247] J.D. Lechleiter, D.E. Clapham, Cell 69 (1992) 283–294.
[248] R.W. Hall, M.O. Thorner, G.L. Mandell, J.A. Sullivan, Y.N. Sinha, D.A. Leong, J. Biol. Chem. 263 (1988) 9682–9685.
[249] A.T. Harootunian, JP.Y. Kao, S. Paranjape, S.R. Adams, B.V.L Potter, R.Y. Tsien, Cell Calcium 12 (1991) 153–164.
[250] B. Hellman, E. Gylfe, E. Grapengiesser, P. Lund, A. Berts, Biochim. Biophys. Acta 113 (1992) 295–305.
[251] G.E. Gylfe, B. Hellman, Arch. Biochem. Biophys. 268 (1989) 404–407.
[252] D.I. Yule, J.A. Williams, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, New York, 1994, pp. 1447–1472.
[253] A. Goldbeter, G. Dupont, M.J. Berridge, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 1461–1465.
[254] G. Dupont, A. Goldbeter, BioEssays 14 (1992) 485–493.
[255] M.J. Berridge, J. Biol. Chem. 265 (1990) 9583–9586.
[256] M. Wakui, B.V.L. Potter, O.H. Petersen, Nature 339 (1989) 317–320.
[257] P. Thorn, O. Gerasimenko, O.H. Petersen, EMBO J. 13 (1994) 2038–2043.
[258] P. Thorn, A.M. Lawrie, P.M. Smith, D.v. Gallacher, O.H. Petersen, Cell 74 (1993) 661–668.
[259] P. Thorn, R. Moreton, M. Berridge, EMBO J. 15 (1996) 999–1003.
[260] Y. Yao, J. Choi, I. Parker, J. Physiol. (London) 482 (1995) 533–553.
[261] P. Camacho, J.D. Lechleiter, Science 260 (1993) 226–229.
[262] C.C.H. Petersen, O.H. Petersen, M.J. Berridge, J. Biol. Chem. 268 (1993) 22262–22264.
[263] S. Girard, D. Clapham, Science 260 (1993) 229–232.
[264] P. Loessberg Stauffer, H. Zhao, K. Luby-Phelps, R.L. Moss, R.A. Star, S. Muallem, J. Biol. Chem. 268 (1993) 19769–19775.
[265] D.I. Yule, E. Stünkel, J.A. Williams, Am. J. Physiol. 271 (1996) C1285–C1294.
[266] S.J. Dixon, D. Stewart, S. Grinstein, S. Spiegel, J. Cell Biol. 105 (1987) 1153–1161.
[267] C. Pothoulakis, R. Sullivan, D. Melnick, G. Triadafilopoulos, A.S. Gadenne, T. Meshulan, J.T. LaMont, J. Clin. Invest. 81 (1988) 1741–1745.
[268] R.J. Gilbert, C. Pothoulakis, J.T. Lamont, M. Yakubovich, Am. J. Physiol. 268 (1995) G487–G495.
[269] B. König, W. Shönfeld, J. Scheffer, W. König, Infect. Immun. 58 (1990) 1591–1599.
[270] C.E. Thomas, D.J. Reed, Hepatology 10 (1989) 375–384.
[271] M. Moore, H. Thor, G. Moore, J. Biol. Chem. 260 (1985) 13035–13040.
[272] S.A. Jewell, G. Bellomo, H. Thor, S. Orrenius, M.T. Smith, Science 217 (1982) 1257–1258.
[273] P.A. Janmey, Annu. Rev. Physiol. 56 (1994) 169–191.
[274] G. Bellomo, F. Mirabelli, P. Crino, G. Finardi, G. Viani, F. Rossi, P. Richelmi, Ann. New York Acad. Sci. 663 (1992) 97–109.
[275] A. Vairengo, P. Nicotera, Comp. Biochem. Physiol. 100C (1991) 81–84.
[276] E.A. Glende Jr., C.K. Pushpendran, Biochem. Pharmacol. 35 (1986) 3301–3307.
[277] M.H. Nathanson, K. Mariwalla, N. Ballatori, J.L. Boyer, Cell Calcium 18 (1995) 429–439.
[278] M.L. Steer, Yale J. Biol. Med. 65 (1992) 421–439.
[279] F.Ch. Mooren, T. Finkes, J. Singh, W. Domschke, Gastroenterology 113 (1997) A276.
[280] W. Zhou, F. Shen, J.E. Miller, Q. Han, M.S. Olson, J. Surg. Res. 60 (1996) 147–155.
[281] J.B. Ward, R. Sutton, S.A. Jenkins, O.H. Petersen, Gastronenterology 111 (1996) 481–491.
[282] A. Meister, M.E. Anderson, Annu. Rev. Biochem. 52 (1983) 711–760.
[283] D. DiMonte, D. Ross, G. Bellomo, L. Elkow, S. Orrenius, Arch. Biochem. Biophys. 235 (1984) 334–342.
[284] H.M. Wong, B.L. Tepperman, Am. J. Physiol. 267 (1994) G1–G9.
[285] F.J.T. Staal, M.T. Anderson, G.E.J. Staal, L.A. Herzenberg, C. Gitler, L.A. Herzenberg, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 3619–3622.
[286] I. Leal-Berumen, P. Conlon, J.S. Marshall, J. Immunol. 152 (1994) 5468–5475.
[287] M. Kubo, R.L. Kincaid, D.R. Webb, J.T. Ransom, Int. Immunol. 6 (1994) 179–188.
[288] P.A. Negulescu, N. Shastri, M.D. Cahalan, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 2873–2877.

[289] Y. Ohmori, T.A. Hamilton, J. Immunol. 148 (1992) 538–545.
[290] R.T. Abraham, S.N. Ho, T.J. Barna, D.J. McKean, J. Biol. Chem. 262 (1987) 2719–2728.
[291] B. Premack, P. Gardner, Am. J. Physiol. 263 (1992) C1119–C1140.
[292] D.G. Raible, E.S. schulman, J. Di Muzio, R. Carillo, T.J. Post, J. Immunol. 148 (1992) 3536–3542.
[293] S. Fiorucci, L. Santucci, P. Gresele, O. Luinetti, A. Morelli, Am. J. Physiol. 268 (1995) G968–G978.
[294] P.N. Bouchelouche, D. Berild, O.H. Nielsen, J. Elmgreen, H.S. Poulsen, Eur. J. Gastroenterol. Hepatol. 7 (1995) 349–356.
[295] N.J. Goulding, P.M. Guyre, Ann. Rheum. Dis. 51 (1992) 594–599.
[296] M.E. Allen, S.P. Young, R.H. Michell, P.A. Bacon, Eur. J. Immunol. 25 (1995) 1547–1554.
[297] K.D. Yang, H. Chen, M. Shaio, Biochem. Biophys. Res. Commun. 211 (1995) 1006–1014.
[298] W. Davis, E.L. Halliwell, S.O. Sage, N.Y. Allen, Cell Calcium 17 (1995) 345–353.
[299] B.s. Bochner, A.A. McKelvey, S.A. Sterbinsky, J.E.K. Hildreth, C.P. Derse, D.A. Klunk, L.M. Lichtenstein, R.P. Schleimer, J. Immunol. 145 (1990) 1832–1837.
[300] M. Uguccioni, M. D'Apuzzo, M. Loetscher, B. Dewald, M. Baggiolini, Eur. J. Immunol. 25 (1995) 64–68.
[301] F. Shibata, K. Knishi, H. Kato, N. Komorita, M. Al-Mokdad, M. Fujioka, H. Nakagawa, Eur. J. Biochem. 231 (1995) 306–311.
[302] A.J. Huang, J.E. Manning, T.M. Bandak, M.C. Ratau, K.R. Hanser, S.C. Silverstein, J. Cell Biol. 120 (1993) 1371–1380.
[303] D.C. Altieri, S.J. Stamnes, C.G. Gahmberg, Biochem. J. 288 (1992) 465–473.
[304] P.D. Arora, J. Ma, W. Min, T. Cruz, C.A.G. McCulloch, J. Biol. Chem. 270 (1995) 6042–6049.
[305] J. Llopis, G.E.N. Kass, S.K. Duddy, G.C. Farell, A. Gahn, S. Orrenius, FEBS Lett. 280 (1991) 84–86.
[306] D.J. McConkey, S.C. Chow, S. Orrenius, M. Jondal, FASEB J. 4 (1990) 2661–2664.
[307] Y. Oshimi, K. Oshimi, S. Miyazaki, J. Physiol. (Lond.) 492 (1996) 319–329.
[308] G. Bellomo, M. Perotti, F. Taddei, F. Mirabelli, G. Finardi, P. Nicotera, S. Orrenius, Cancer Res. 52 (1992) 1342–1346.
[309] G.W. de Vries, A. McLaughlin, M.B. Wenzel, J. Perez, D. Harcourt, G. Lee, M. Garst, L.A. Wheeler, Inflammation 19 (1995) 261–275.
[310] A. Fleckenstein, J. Janke, H.J. Döring, O. Leder (Eds.), Recent Advances in Studies of Cardiac Structure and Metabolism, Vol. 6, Park Press, Baltimore, 1975, pp. 21–32.
[311] W.R. Jacobs, M. Sgambati, G. Gomez, P. Vilaro, M. Higdon, P.D. Bell, L.J. Mandel, Am. J. Physiol. 260 (1991) C545–C554.
[312] W. Rouslin, J. Biol. Chem. 258 (1983) 9657–9661.
[313] A. Yatani, M. Goto, Jpn. J. Physiol. 33 (1983) 403–415.
[314] A.D. Sharma, J.E. Saffiz, B.I. Lee, B.E. Sobel, J. Clin. Invest. 72 (1983) 802–818.
[315] G.J. Rozanski, R.C. Witt, Am. J. Physiol. 267 (1994) H1361–H1367.
[316] M. Tani, J.R. Neely, J. Mol. Cell. Cardiol. 22 (1990) 57–72.
[317] J.I. Goldhaber, Am. J. Physiol. 271 (1996) H823–H833.
[318] A. Fleckenstein, M. Frey, G. Fleckenstein-grün, Eur. Heart J. 5 (1993) 43–50, Suppl. H.
[319] G.E. Billmann, B. McIlroy, J.D. Johnson, FASEB J. 5 (1991) 2586–2592.
[320] J.K. Gwathmey, L. Copelas, R. MacKinnon, F.J. Schoen, M.D. Feldman, W. Grossman, J.P. Morgan, Circ. Res. 61 (1987) 70–76.
[321] J.P. Morgan, R.E. Erny, P.D. Allen, W. Grossman, J.K. Gwathmey, Circulation 8 (1990) III21–III32.
[322] M.A. Movsesian, M.R. Bristow, J. Krall, Circ. Res. 65 (1989) 1141–1144.
[323] M.A. Movsesian, J. Colyer, J.H. Wang, J. Krall, J. Clin. Invest. 85 (1990) 1698–1702.
[324] M. Arai, N.R. Alpert, D.H. MacLennan, P. Barton, M. Periasamy, Circ. Res. 72 (1993) 463–469.
[325] M. Flesch, R.H.G. Schwinger, P. Schnaber, F. Schiffer, I. can Gelder, U. Bavendieck, M. Südkamp, F. Kuhn-Regnier, M. Böhm, J. Mol. Med. 74 (1996) 321–332.
[326] A. Brillantes, P. Allen, T. Takahashi, S. Izumo, A.R. Marks, Circ. Res. 71 (1992) 18–26.
[327] T. Studer, H. Reinecke, J. Bilger, T. Eschenhagen, M. Böhm, G. Hasenfuss, H. Just, J. Holtz, H. Drexler, Circ. Res. 75 (1994) 443–453.
[328] A.R. Marks, M.R. Taubmann, A. Saito, Y. Dai, S. Fleischer, J. Cell Biol. 114 (1991) 303–312.
[329] H. Shih, M.S. Walthen, H.B. Marshall, J.M. Caffrey, M.D. Schneider, J. Clin. Invest. 85 (1990) 781–789.
[330] C.B. Neylon, S.M. Bryant, P.J. Little, A. Bobik, Biochem. Biophys. Res. Commun. 204 (1994) 678–684.
[331] C.M. Thaik, A. Calderone, N. Takahashi, W.C. Colucci, J. Clin. Invest 96 (1995) 1093–1099.
[332] M.P. Blaustein, T. Ashida, W.F. Goldman, W.G. Wier, J.M. Hamlyn, Ann. New York Acad. Sci. 488 (1986) 199–217.
[333] B. Johansson, A.P. Somlyo, in: D.F. Bohr, A.P. Somlyo, H.V. Sparks (Eds.), Handbook of Physiology: Vascular Smooth Muscle, American Physiological Society, Bethesda, MD, 1980, pp. 301–324.
[334] G. Bruschi, M.E. Bruschi, M. Caroppo, G. Orlandini, C. Pavarani, A. Cavatorta, Life Sci. 5 (1984) 535–542.
[335] A.C. Shore, G.W. Beynon, J.C. Jones, N.D. Markandu, G.A. Sagnelles, G.A. MacGregor, J. Hypertens. 3 (1985) 183–187.
[336] L.A. Jelicks, R.K. Gupta, J. Biol. Chem. 265 (1990) 1394–1400.
[337] F.R. Bühler, T.J. Resink, Am. J. Hypertens. 1 (1988) 42–46.
[338] P. Erne, P. Bolli, F.R. Bühler, New Engl. J. Med. 310 (1984) 1084–1087.

[339] J.A. Metz, L. Roberts, M. McClung, C. Morris, D.A. McCarron, J. Am. Soc. Nephrol. 4 (1993) 699.
[340] D.A. McCarron, P.A. Pingree, R.J. Rubin, S.M. Gaucher, M. Molitch, S. Krutzik, Hypertension 2 (1980) 162–168.
[341] N.D. Papagalanis, P. Skopelitis, A. Kourti, G. Kostogianni, A. Karabatsos, M. Gennadioum, S. Thomas, M. Sanartzis, T. Mountokalakis, Nephron 59 (1991) 226–231.
[342] N.D. Papagalanis, A. Kourti, A. Tolis, P. Skopelitis, A. Karabatsos, G. Kostayami, M. Gunnadeon, M. Elisof, T. Mountokalakis, Am. J. Hypertens. 6 (1993) 59–65.
[343] P. Strazullo, V. Nunziata, M. Cirillo, R. Giannattasio, L.A. Ferrara, P.L. Mattioli, A. Mancini, Clin. Sci. 65 (1983) 137–141.
[344] E.W. Young, T. Drücke, D.A. McCarron, Miner. Electrolyte Metabol. 16 (1990) 154–158.
[345] E.W. Young, C.D. Morris, D.A. McCarron, J. Lab. Clin. Med. 120 (1992) 624–632.
[346] J.M. Elizan, J. Villar, A. Zalzar, L. Rojas, D. Chan, G.F. Bryce, Am. J. Obstet. Gynecol. 146 (1983) 175–180.
[347] N. Kawasaki, K. Matsui, M. Ito, T. Nakamura, T. Yoshimura, H. Ushijima, M. Maevama, Am. J. Obstet. Gynecol. 152 (1985) 576–582.
[348] J.A. Cutler, E. Brittain, Am. J. Hypertens. 3 (1990) 137S–146S.
[349] H. Mikami, T. Ogihara, Y. Tabuchi, Am. J. Hypertens. 3 (1990) 147S–151S.
[350] D.E. Grobbee, H.J. Waal-Manning, Drugs 39 (1990) 7–18.
[351] D.A. McCarron, New Engl. J. Med. 307 (1982) 226–228.
[352] C.M. Roullet, E.W. Young, J.-B. Roullet, B. Lacour, T. Drüeke, D.A. McCarron, Am. J. Physiol. 257 (1989) F574–F579.
[353] C.M. Roullet, J.-B. Roullet, A.-S. Martin, D.A. McCarron, Am. J. Hypertens. 7 (1994) A1203.
[354] C. Lugnier, B. Ilien, J.C. Stoclet, Eur. J. Pharmacol. 53 (1978) 109–112.
[355] J. Higaki, T. Ogihara, Y. Kumahara, E.L. Bravo, Clin. Sci. 68 (1985) 407–410.
[356] L. Fliegel, M.P. Walsh, D. Singh, C. Wong, A. Barr, Biochem. J. 282 (1992) 139–145.
[357] B. Bertrand, S. Wakabayashi, T. Ikeda, J. Pouyssegur, M. Shigekawa, J. Biol. Chem. 269 (1994) 13703–13709.
[358] L. Tobian, J.T. Binion, Circulation 5 (1952) 745–758.
[359] P.J. Hilton, New Engl. J. Med. 314 (1986) 222–229.
[360] M.P. Blaustein, Am. J. Physiol. 232 (1977) C165–C173.
[361] W.H. Beierwalter, W.J. Arendshorst, P.J. Klemmer, Hypertension (Dallas) 4 (1982) 908–915.
[362] L.C. Garg, N. Narang, S. McArdle, Am. J. Physiol. 249 (1985) F863–F869.
[363] J.M. Hamlyn, M.P. Blaustein, Am. J. Physiol. 251 (1986) F563–F575.
[364] T.J. Resink, V.A. Tkachuk, P. Erne, Hypertension 8 (1985) 159–163.
[365] M.A. Miller, G.A. Sagnella, N.D. Markandu, G.A. MacGregor, J. Hypertens. 12 (1994) 929–938.
[366] M. Juchaszova, H. Shimizu, M.L. Borin, R.K. Yip, E.M. Santiago, G.E. Lindenmayer, M.P. Blaustein, Ann. New York Acad. Sci. 779 (1996) 318–337.
[367] M.P. Blaustein, Am. J. Physiol. 264 (1993) C1367–C1387.
[368] G. Rossi, P. Manunta, J.M. Hamlyn, E. Pavan, R. DeToni, A. Semplicini, A.C. Pessina, J. Hypertens. 13 (1995) 1181–1191.
[369] A. Rothstein, Rev. Physiol. Biochem. Pharmacol. 112 (1989) 235–257.
[370] C.R.K. Dudley, D.J. Taylor, L.L. Ng, G.J. Kemp, P.J. Ratcliffe, G.K. Radda, J.G.G. Ledingham, Clin. Sci. 79 (1990) 491–497.
[371] D. Rosskopf, R. Düsing, W. Siffert, Hypertension 21 (1993) 607–617.
[372] D. Rosskopf, E. Frömter, W. Siffert, J. Clin. Invest. 92 (1993) 2553–2559.
[373] W. Siffert, R. Düsing, Hypertension 26 (1995) 649–655.
[374] J.M. Hamlyn, B.P. Hamilton, P. Manunta, J. Hypertens. 14 (1996) 151–167.
[375] M.P. Blaustein, Kidney Int. 49 (1996) 1–6.
[376] W.R. Mathews, D.W. DuCharma, J.M. Hamlyn, D.W. Harris, F. Mandel, M.A. Clark, S.H. Ludenz, Hypertension 17 (1991) 930–935.
[377] W.R. Mathews, J.H. Ludens, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 6259–6263.
[378] C.M. Yuan, P. Manunta, J.M. Hamlyn, S. Chen, E. Bohen, J. Yeun, F.J. Haddy, M.B. Pamnani, Hypertension 22 (1993) 178–187.
[379] P. Manunta, A.C. Rogowski, B.P. Hamilton, J.M. Hamlyn, J. Hypertens. 12 (1994) 549–560.
[380] R. Zimlichman, D.S. Goldstein, S. Zimlichman, H.R. Keister, J. Hypertens. 5 (1987) 605–609.
[381] R.J. Miller, Prog. Neurobiol. 37 (1991) 255–285.
[382] R.C. Malenka, J.A. Kauer, D.J. Perkel, R.A. Nicoll, Trends Neurosci. 12 (1989) 444–450.
[383] S. Orrenius, P. Nicotera, J. Neural Transm. 43 (1994) 1–11, Suppl.
[384] D.W. Choi, Science 258 (1992) 241–243.
[385] B.K. Siesjö, Magnesium 8 (1989) 223–237.
[386] J.A. Connor, W.J. Wadman, P.E. Hockberger, R.K. Wong, Science 240 (1988) 649–653.
[387] H. Manev, M. Favaron, A. Guidotti, E. Costa, Mol. Pharmacol. 36 (1989) 106–112.
[388] J.A. Strong, A.P. Fox, R.W. Tsien, L.K. Kaczmarek, Nature 325 (1987) 714–717.
[389] C. Wahlestedt, E. Golanov, S. Yamamoto, F. Yee, H. Ericson, C.E. Inturrisi, D.J. Reis, Nature 363 (1993) 260–263.
[390] B.K. Siesjö, Eur. Neurol. 30 (1990) 3–9.
[391] P.K. Stys, S.G. Waxman, B.R. Ransom, Ann. New York Acad. Sci. 639 (1991) 329–332.
[392] H. Tsubokawa, K. Oguro, H.P.C. Robinson, T. Masuzuwa, T.S.G. Rhee, T. Takenawa, N. Kawai, Neuroscience 59 (1994) 291–297.
[393] H. Tsubokawa, K. Oguro, H.P.C. Robinson, T. Masuzawa, N. Kawai, J. Physiol. (London) 497 (1996) 67–78.

[394] H.E. Scharfman, P.a. Schwartzkroin, Science 246 (1989) 257–260.
[395] C.W. Heizmann, K. Braun, Trends Neurosci. 15 (1992) 259–264.
[396] K. Kato, F. Suzuki, N. Kurobe, K. Okajma, N. Ogasawara, M. Nagaya, T. Yamanaka, J. Mol. Neurosci. 2 (1990) 109–113.
[397] A.M. Iacopino, S. Christakos, J. Biol. Chem. 265 (1990) 10177–10180.
[398] D.R.C. McLachlan, L. Wong, C. Bergeron, K.G. Baimbridge, Alzheimer Dis. Assoc. Disord. 1 (1987) 171–179.
[399] W.S. Griffin, L.C. Stanley, C. Ling, L. White, V. MacLead, L.J. Perrot, C.L. White, C. Araoz, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 7611–7615.
[400] C. Peterson, R. Ratan, M. Shelanski, J. Goldman, Ann. New York Acad. Sci. 568 (1989) 263–270.
[401] R. Joseph, E. Han, Biochem. Biophys. Res. Commun. 184 (1992) 1441–1447.
[402] H. Hartmann, A. Eckert, W.E. Müller, Biochem. Biophys. Res. Commun. 194 (1993) 1216–1220.
[403] E. Ito, K. Oka, R. Etcheberrigaray, T.J. Nelson, D.L. McPhie, B. Tofel-Grehl, G.E. Gibson, D.L. Alkon, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 534–538.
[404] M.P. Mattson, B. Cheng, D. Davis, K. Bryant, I. Lieberburg, R.E. Rydel, J. Neurosci. 12 (1992) 376–389.
[405] N. Arispe, E. Rojs, H.B. Pollard, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 567–571.